Studii şi Cercetări Ştiinţifice 2020, **21** (3), pp. 395 – 405
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

IMPROVEMENT OF THE POLYPHENOLIC CONTENT, ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITIES OF *CORNUS OFFICINALIS* FRUIT BY *BIFIDOBACTERIUM BIFIDUM* FERMENTATION

Xiuren Zhou[1*], Yimin Zhao[2], Yongchao Li[1], Guifang Xu[1], Xiangxu Zhou[3]

[1]Henan Institute of Science and Technology/ School of Life Science and Technology, Hualan Road 90, Xinxiang, China
[2]Guangxi Botanical Garden of Medicinal Plants, Changgang Road 189, Nanning, China
[3]Affiliated Middle School of Henan Normal University, Jianshedonglu 85, Xinxiang, China

*Corresponding Author: xiuren_zhou@yahoo.com

Received: August, 26, 2019
Accepted: September, 21, 2020

Abstract: Fermentation is considered a promising way to increase the bioactive substance content as well as the curing efficacy of foods and herbal medicines. However, in the vast majority of cases, it remains unknown what bioactive ingredient and physiological activity are altered in traditional herbs after fermentation. Here, in order to find the effect of fermentation on the bioactive compound content and health efficacy of *Cornus officinalis* fruit, an important Chinese traditional medicine, its total phenolic content, total flavonoid content, antioxidant activity and anti-tyrosinase activity were estimated during *Bifidobacterium bifidum* fermentation process. The results showed that, during COF (*Cornus officinalis* fruit)'s fermentation, the total phenolic contents were significantly increased, however, the total flavonoid contents weren't obviously changed, and that the antioxidant activity as well as the anti-tyrosinase activity was enhanced. In addition, the total phenolic content was positively related not only to the antioxidant activity but also to the anti-tyrosinase activity. This research can help us better understand the alterations of ingredients and physiological activities of fermented traditional medicines that influence curing efficacy.

Keywords: *active compound, biochemical and physiological activity, functional food, increase, microorganism, traditional herbal medicine, zymosis*

INTRODUCTION

Fermentation is a promising approach through which curing effects and bioactive ingredients of traditional Chinese medicines and functional foods may be improved. Traditional East-Asian fermented foods, such as Chinese fermented soya bean, Korean soy sauce koji and Japanese natto, obtain higher nutritional value and more health effects through fermentation processes. Currently, some studies have confirmed that fermentation can significantly strengthen or increase curing efficacy or bioactive composition proportion of herbal medicine. When *Phellinus linteus* was fermented with other eight herbal medicines, the culture broth could suppress melanogenesis and microphthalmia-associated transcription factor, and had a high phenolic content [1]. After a fermentation process, several herbs including *Viola mandshurica* [2], red ginseng [3], *Rhodiola rosea, Lonicera japonica* [4], walnut, Moutan Cortex Radicis and asparagus root [5] showed improved anti-melanogenesis, anti-aging, tyrosinase inhibitory or antioxidative activities. As discovered by Suh et al., the fermented mixture of *Cudrania tricuspidata*, *Lonicera caerulea* and soybean have increased antioxidant and pancreatic-lipase-inhibitory activities [6]. Fermented herbs or traditional herb formulations could enhance anti-inflammatory, immunity or immunomodulatory ability, as published by some researchers [7 – 14]. All described above have demonstrated that fermentation could increase the bioactive substance content of herbal medicine, functional food and other food and strengthen their healthy or curing efficacy.

COF (*Cornus officinalis* fruit), a traditional medicine in China, is applied to treat liver and kidney disease. It has been discovered that the fruit could inhibit cancer [15 – 17] and reduce blood glucose concentration [18 – 21]. It has also indicated that this traditional medicine has antioxidant [22 – 24] and cognitive-enhancing [25] capacities and enhances erectile function [26]. A few studies have been involved in the fermentation of the traditional Chinese herb. Park et al. revealed that fermented COFs could improve hair growth [27]. Some investigators detected fermentation conditions and determination of loganin during COF health wine production [28]. Moreover, extraction of ursolic acid from this herb was investigated on the condition of fermentation [29]. However, alteration of bioactive substance content, antioxidant and anti-tyrosinase activities is still unclear in fermented COFs, which may play a crucial role in a better understanding of curing efficacies or other effects of the herb.

Bifidobacterium bifidum is one of the most important probiotic bacteria human digestive tract, which can reduce the chances of acute diarrhea and the risk of harmful bacterium infections, and contributes to the maintenance of intestinal microbial balance. So, in this present paper, the effects of *B. bifidum* fermentation and fermentation duration on the biochemical composition and activity of COF were studied. Phenolic content, flavonoidic content, antioxidant capacity and anti-tyrosinase capacity were determined in fermented COFs and unfermented COFs. The results demonstrate that fermentation can alter the phenolic concentration of COF, and that it can improve this herb's antioxidant and anti-tyrosinase ability. Those findings will help people to better understand the mechanism underlying improved curing efficacy of fermented COFs.

MATERIALS AND METHODS

Herbal material and pretreatment

Fresh COFs were collected from a *Cornus officinalis* cultivation base belonging to Zhongjing Wanxi Pharmaceutical Co., Ltd. in June 2015. The nucleuses were removed from those fruits. Then, the flesh and rice wine (w : w = 3 : 1) was mixed and slowly rubbed. The rubbed flesh was steamed for three hours and then was dried at 60 °C in an oven for 72 hours. The dried flesh was ground into powder whose particle diameter should be less than 3 mm. The powder was prepared for subsequent fermentation and extraction.

Regents

Tyrosine, tyrosinase, tyrosinase inhibitor, ascorbic acid, arbutin, DPPH (1,1-diphenyl-2-picrylhydrazyl) were purchased from Sigma-Aldrich Co.. Folin-Ciocalteu reagent, aluminum chloride, sodium nitrite and sodium carbonate were bought from Sangon Biotech (Shanghai) Co. Ltd.. *B. bifidum* strains were obtained from Shangchuan Biotech Co. Ltd. All experimental chemicals used were of analytical grade, and the water applied was double-distilled water.

Fermentation process of COF

500 mL flasks containing 180 mL of liquid fermentation medium were added 0, 0.6, 1.2, 2.4 and 4.8 g of dried powder of COF as described above. One liter of liquid fermentation medium contained 10.0 g peptone, 8.0 g beef extract powder, 4.0 g yeast extract, 20.0 g glucose, 1 mL sorbitan mono-oleate, 2.0 g dipotassium hydrogen phosphate, 5.0 g sodium acetate, 2.0 g triammonium citrate, 0.2 g manganese sulphate heptahydrate. Next, the *p*H of liquid medium in each flask was adjusted to 7.0 by adding sodium hydroxide or hydrochloric acid solution, and then 200 mL final volume was achieved by adding medium. These flasks were sterilized in an autoclave at 121 °C for 30 minutes. Under a sterilized condition, all sterilized flasks were inoculated with 3 mL of 1×10^7 cfu·mL^{-1} *B. bifidum* culture. Afterward, those inoculated mixtures were statically cultured at 38 °C in an anaerobic incubator (YQX-11, LNB Instrument, China) for different duration: 0, 6, 12, 18, 24, 30, 36 or 42 hours. The fermentation process of the samples was terminated by placing them in a 0 - 4 °C environment. The samples with 0 g of COF were used negative control. All samples were carried out in triplicate.

Extraction of total phenols and total flavonoids

The fermentation broth was centrifuged at 1000 g for 30 minutes, and then 100 mL of supernatant was collected and evaporated under reduced pressure in a rotary evaporator until a light yellow paste was obtained. The paste was repeatedly extracted with 100 mL petroleum ether in order to remove the lipids. Then, repeatedly extracted the residue with 100 mL of 65 % methanol at 60 °C under an ultrasound condition for 120 minutes,

took supernatant every time. Afterward, the supernatant taken twice were mixed for the following assays.

Determination of total phenolic content

The total phenolic content was evaluated according to an improved Folin-Ciocalteu method. 0.4 mL of experimental sample was added into a 10 mL test tube which contained 5.2 mL of water. Vortexed the tubes for 1 minutes, and then added 0.4 mL of Folin-Ciocalteu's reagent into each tube. Shake the tubes on a vortex oscillator again for 1 minute. After 4 minutes, 4 mL of 10 % Na_2CO_3 was added into each test tubes. After that, shake these tubes vigorously for 30 seconds and then made them stand at room temperature for 1 hour. Afterward, the absorbance of each sample was determined against a blank at 746 nm with a spectrophotometer (Unocal UV-2000, Unocal Corporation, United States). 0.050, 0.100, 0.150, 0.300, 0.600 and 1.200 $g \cdot L^{-1}$ gallic acid solutions were used standard solutions to construct a calibration curve. The total phenolic content is expressed as milligrams of gallic acid per gram of dry substance.

Determination of the total flavonoid content

The determination of total flavonoid content of the extract was carried out according to a modified method published by Liu et al. (2005) [30]. 0.4 mL test sample was added into a 5 mL volumetric flash containing 5 mL of 30 % ethanol. The flashes were shook, and then stand for 5 minutes. 10 % Al_3NO_3 solution was added in the flashes and shook well. After 6 minutes, 2 mL of 1.0 $mol \cdot L^{-1}$ NaOH solution was added in the flash, and the solution volume was made up to 10 mL with 30 % ethanol. The flashes were shook gently in order to mix different ingredient thoroughly, next, the flashes were left for 15 minutes. The absorbance of the solution was measured against a blank at 510 nm using a spectrophotometer (Unocal UV-2000, Unocal Corporation, United States). Applied 0.050, 0.100, 0.150, 0.300, 0.600, 1.200 $g \cdot L^{-1}$ rutin as a standard to construct a calibration curve. The total flavonoid content was shown as milligram of rutin equivalents per gram dry matter.

Determination of the free radical scavenging activity

The free radical scavenging activity of the extract was estimated through a modified version of the DPPH method as described by Brand-Williams et al (1995) [31]. A 0.2 mL of test sample was added in a tube containing 4 mL of 0.2 mM DPPH dissolved in methanol and added to 10 mL with methanol. The tubes were vortexed for 1 minute and then placed in a test tube rack for 30 minutes. The absorbance of the solution were determined was recorded using a spectrophotometer (Unocal UV-2000, Unocal Corporation, United States) at 517 nm. The free scavenging activity was expressed as the free radical scavenging percentage (K) based on the following equation:

$$K = [(A_0\text{-}A)/A_0]*100\% \quad (1)$$

where K means free radical scavenging percentage, A_0 is absorbance value of control solution and A is absorbance of sample solution. The DPPH solution without test sample was used as control. Ascorbic acid was positive control.

Measuring of anti-tyrosinase activity

Anti-tyrosinase activity of the extract was assayed using a improved version of Hearing's method [32]. A 4 mL of 2.5 mM levodopa was added to a 2 mL of 0.05 M phosphate buffer (*p*H=6.8), and then mixed well. 1 mL of assayed sample and 3 mL of tyrosinase (100 $U \cdot mL^{-1}$) were added into that mixture. The solutions were incubated for 15 minutes at 30 °C. The termination of enzyme reaction was perfected by placing the reaction systems in an ice bath. The absorbance of the system was determined with a spectrophotometer (Unocal UV-2000, Unocal Corporation, United States) at 490 nm. The anti-tyrosinase activity was expressed as tyrosinase inhibition percentage (R) based on the following equation:

$$R = \{[(A_1-A_2)-(B_1-B_2)]/(A_1-A_2)\}*100\% \quad (2)$$

where R represents tyrosinase inhibition percentage, A_1 is the absorbance value of control system with enzyme, A_2 denotes the absorbance value of control system without enzyme, B_1 means the absorbance value of sample system with enzyme, and B_2 indicates the absorbance value of sample system without enzyme. Beta-arbutin was positive control.

Statistical analysis

Data were tested for normality and variance constancy before statistical analysis. One-way ANOVA and LSD multiple comparison tests were applied to find differences between groups in active compound contents or physiological-biochemical activities. Correlations between active compound and physiological-biochemical index were made by linear regression analysis. Mean differences were considered statistically significant when $P < 0.05$. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Results are presented in the form mean.

RESULTS AND DISCUSSION

Changes of the total phenolic and flavonoid content during the fermentation process

After fermentation, the total phenolic contents of test samples significantly increased, however, the sample had no significant alteration in the total flavonoidic contents. As shown in Figure 1, the total phenolic content of culture broths containing different amounts of COF increased with the extension of fermentation time (Figure 1a), although the broths not inoculated with *B. bifidum* showed no alteration in the total phenolic levels over time (Figure 1b). Moreover, Figure 1a also indicates that the increase of the total phenolic contents during fermentation for 12, 18, 24 hours significantly increased. It was similar to the results discovered in the studies on other fermented traditional foods or herbs [1, 5, 33 – 35]. In contrast to the total phenolic, the total flavonoid content had no statistically significant alteration during fermentation (Figure 2a) which was similar to the total flavonoid level in COF broth not fermented by *B. bifidum* (Figure 2b). It was different from the conclusions of other researchers that

fermentation usually decreases the total flavonoid contents of substrate because major compounds composed of total flavonoid are oxidized during the process [35 – 38]. This distinction may be likely due to differences in the types of fermenting microorganisms or/and substrates. These discoveries provide a suggestion that the fermentation may be a better way to promoting the phenolic content of traditional food and medicine, however, it may not be suitable for increasing the flavonoid content of certain traditional foods or medicines.

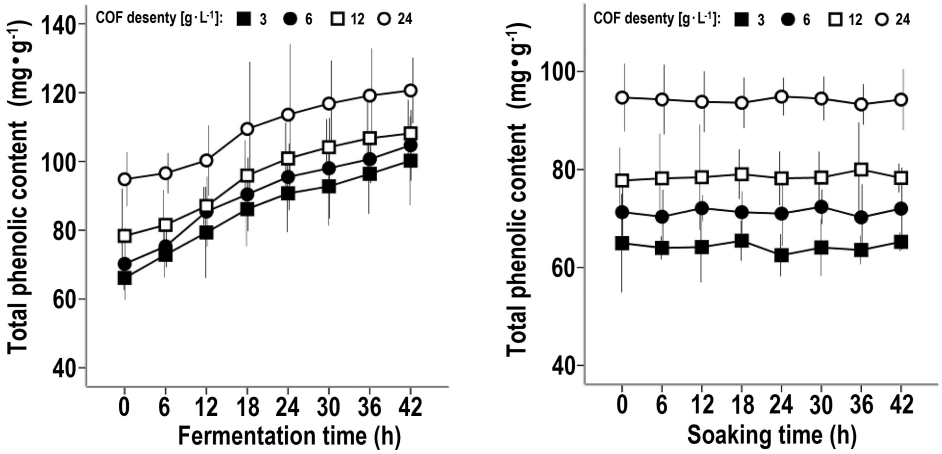

Figure 1. *Total phenolic content of B. bifidum fermentation broth (a) and control (no fermentation) (b) at different COF densities*

**error bars represent standard deviation of three independent experiment*

**total phenolic content was expressed as milligrams of gallic acid per equivalents gram of dry matter*

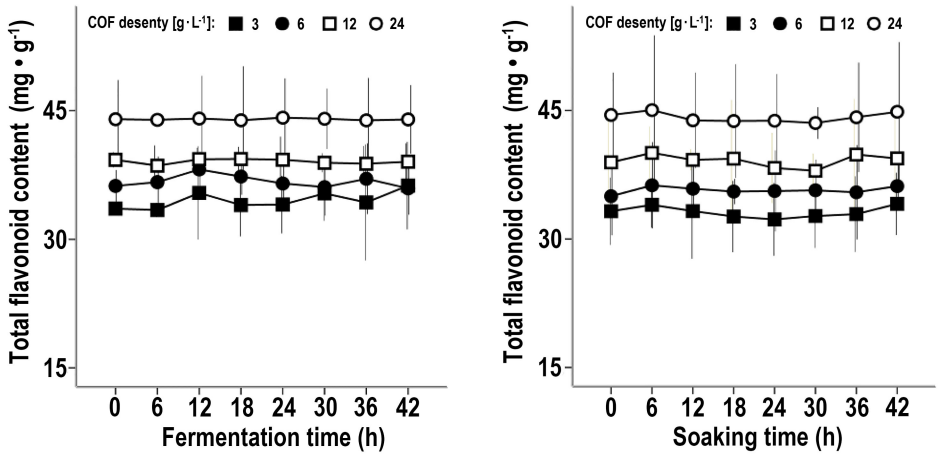

Figure 2. *Total flavonoid content of B. bifidum fermentation broth (a) and control (no fermentation) (b) at different COF densities*

**error bars represent standard deviation of three independent experiment*

**total flavonoid content was shown as milligram of rutin equivalents per gram dry matter*

Changes of the antioxidant and anti-tyrosinase activities during the fermentation process

Both the antioxidant and anti-tyrosinase activity of the fermented COF were significantly improved. Figure 3 shows that the antioxidant activities of COF fermented for 12, 18, 24 hours were significantly enhanced, although those of COF fermented for 30, 36, 42 hours were not significantly altered. Like the antioxidant, there was a significant exaltation in the anti-tyrosinase activity during fermentation for 12, 18, 24 hours (Figure 4). Here, both the antioxidant and anti-tyrosinase activity of COF were improved during fermentation. Some previous researches have shown that fermentation can not only strengthen the antioxidant activity of traditional foods and medicines, but also their anti-tyrosinase activity [4, 5, 35]. It is demonstrated that microbial fermentation may partly rely on the same or similar mechanism to increase the antioxidant and anti-tyrosinase activity of substrates.

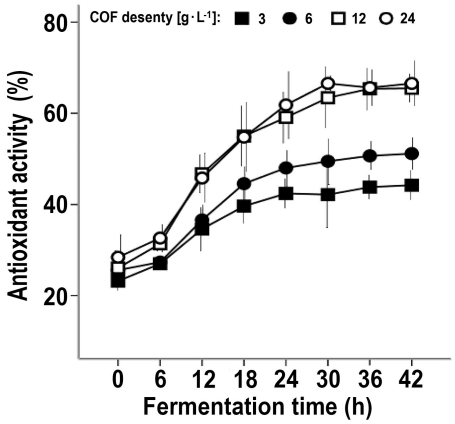

Figure 3. *Antioxidant activity (%) of B. bifidum fermentation broth of at different COF densities*

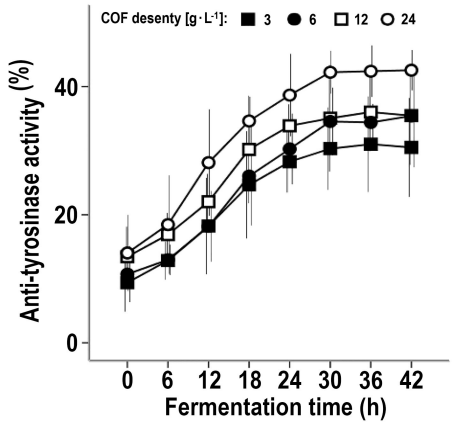

Figure 4. *Anti-tyrosinase activity (%) of B. bifidum fermentation broth of at different COF densities*

Relationship between the antioxidant or anti-tyrosinase activity and the total phenol or flavonoid content

In various COF fermentation times at the same COF density, the correlation between the antioxidant activity and the total phenolic content was strongly positive, while that between the antioxidant activity and the flavonoid content was poor. As shown in Table 1, these correlation coefficient values of the antioxidant activities and the total phenolic contents were between 0.959 and 0.985, indicating a strong relationship between them. This result supported the discoveries that the antioxidant activity of some traditional medicines and foods may be improved through the increasement of the phenolic content by fermentation [5, 35, 40]. In contrast, the relationship between the antioxidant activity and the total flavonoid content was very insufficient on the same conditions, as suggested by the low correlation coefficient values (0.012-0.497) (Table 1). The poor relationship between the antioxidant activity and the flavonoid content was likely because of no obvious alteration of flavonoid content during fermentation process.

Table 1. *Correlation coefficient (R) of the bioactive substances and the physiological activities at various fermentation times of different COF densities*

COF density [g·L^{-1}]	Total phenolic content vs antioxidant activity	Total flavonoid content vs antioxidant activity	Total phenolic content vs anti-tyrosinase activity	Total flavonoid content vs anti-tyrosinase activity
3	0.985	0.497	0.982	0.565
6	0.959	0.048	0.973	-0.086
12	0.979	0.012	0.987	-0.029
24	0.977	0.089	0.973	0.061

The correlation coefficient values between the anti-tyrosinase activity and the total phenolic content was between 0.973 and 0.987 at different fermentation time at various COF concentrations (Table 1), suggesting that the increasement of this compound content may strengthen the anti-tyrosinase activity after fermentation. It was similar to the speculation that fermentation may positively influence the anti-tyrosinase activity of food and herbs by increasing their phenolic content [5, 35, 39]. In contrast, the correlation coefficient values between anti-tyrosinase activity and flavonoid was between -0.086 and 0.565 (Table 1), indicating that flavonoid may not be involved in the anti-tyrosinase activity in this research. No relationship between anti-tyrosinase activity and flavonoid likely because of little alteration in the flavonoid content during fermentation.

In general terms, the COF fermented by the probiotic *B. bifidum* exhibited the increasement of the total phenolic content, the enhancement of the antioxidant activity, and the improvement of anti-tyrosinase activity. Moreover, it was demonstrated that the antioxidant and anti-tyrosinase activities were positively related with the phenolic content during fermentation process. Therefore, it was confirmed that fermentation had the ability to promote the content of certain bioactive compounds and to improve the beneficial health effects in COF. Additionally, it may be feasible to speculate that when COF is eaten by people with a normal gut microbiota, its bioactive compounds and beneficial health effects will be improved, owing to gut probiotic fermentation.

However, more research may be needed to determine what type of phenol is altered and to clarify whether there are significant changes in other active ingredients under the condition of fermentation.

CONCLUSIONS

Currently, it has been believed that fermentation plays a very hopeful role in promoting bioactive compound contents and health efficacies of traditional medicines and foods. Although COF, an important traditional Chinese herbal medicine, has been sporadically studied in the condition of fermentation, the effects of fermentation on the bioactive substance contents and curing efficacies of this herb still remain unknown. Here, during COF's fermentation, it has been discovered that the total phenolic contents were significantly increased, although the total flavonoid contents were obviously changed, and that the antioxidant activity as well as the anti-tyrosinase activity was enhanced. Furthermore, the total phenolic contents were not only positively related to the antioxidant activity but also the anti-tyrosinase activity. Therefore, it is feasible to increase bioactive product contents of foods or herbal medicines and to improve their health effects using fermentation. However, more research may be needed to determine what type of phenol is altered and to clarify whether there are significant changes in other active ingredients under the condition of fermentation.

ACKNOWLEDGMENTS

This research was funded by Science and Technology Department of Henan Province. The authors thank Junke Li for their hospitability and sample collection.

REFERENCES

1. Cha, J.Y., Yang, H.J., Moon, H.I., Cho, Y.S.: Inhibitory effect and mechanism on melanogenesis from fermented herbal composition for medical or food uses, *Food Research International*, **2012**, **45** (1), 225-231;
2. Kwak, Y.J., Kim, K.S., Kim, K.M., Yu, H.Y., Chung, E., Kim, S.J.: Fermented *Viola mandshurica* inhibits melanogenesis in B16 melanoma cells, *Bioscience, Biotechnology, and Biochemistry*, **2011**, **75** (5), 841-847;
3. Lee, H.S., Kim, M.R., Park, Y., Park, H.J., Chang, U.J., Kim, S.Y., Suh, H.J.: Fermenting red ginseng enhances its safety and efficacy as a novel skin care anti-aging ingredient: in vitro and animal study, *Journal of Medicinal Food*, **2012**, **15** (11), 1015-1023;
4. Chen, Y.S., Liou, H.C., Chan, C.F.: Tyrosinase inhibitory effect and antioxidative activities of fermented and ethanol extracts of *Rhodiola rosea* and *Lonicera japonica*, *The Scientific World Journal*, **2013**, **2013**, ID 612739;
5. Wang, G.H., Chen, C.Y., Lin, C.P., Huang, C.L., Lin, C., Cheng, C.Y., Chung, Y.C.: Tyrosinase inhibitory and antioxidant activities of three *Bifidobacterium bifidum*-fermented herb extracts, *Industrial Crops and Products*, **2016**, **89**, 376-382;
6. Suh, D.H., Jung, E.S., Park, H.M., Kim, S.H., Lee, S., Jo, Y.H., Lee, M.K., Jung, G., Do, S.G., Lee, C.H.: Comparison of metabolites variation and antiobesity effects of fermented versus nonfermented mixtures of *Cudrania tricuspidata*, *Lonicera caerulea*, and soybean according to fermentation in vitro and in vivo, *PloS One*, **2016**, **11**, e0149022;

7. Joo, S.S., Won, T.J., Nam, S.Y., Kim, Y.B., Lee, Y.C., Park, S.Y., Park, H.Y., Hwng, K.W., Lee, D.I.: Therapeutic advantages of medicinal herbs fermented with *Lactobacillus plantarum*, in topical application and its activities on atopic dermatitis, *Phytotherapy Research*, **2009**, **23** (7), 913-919;
8. Lee, W.W., Ahn, G., Arachchillage, J.P., Kim, Y.M., Kim, S.K., Lee, B.J., Jeon, Y.J.: A polysaccharide isolated from *Ecklonia cava* fermented by *Lactobacillus brevis* inhibits the inflammatory response by suppressing the activation of nuclear factor-κB in lipopolysaccharide-induced RAW 264.7 macrophages, *Journal of Medicinal Food*, **2011**, **14** (12), 1546-1553;
9. Kim, M.S., Kim, W.G., Chung, H.S., Park, B.W., Ahn, K.S., Kim, J.J., Bae, H.: Improvement of atopic dermatitis-like skin lesions by *Platycodon grandiflorum* fermented by *Lactobacillus plantarum* in NC/Nga mice, *Biological and Pharmaceutical Bulletin*, **2012**, **35** (8), 1222-1229;
10. Bose, S., Kim, H.: Evaluation of in vitro anti-inflammatory activities and protective effect of fermented preparations of Rhizoma Atractylodis Macrocephalae on intestinal barrier function against lipopolysaccharide insult, *Evidence-Based Complementary and Alternative Medicine*, **2013**, **2013**, ID 363076;
11. Lee, W.W., Ahn, G., Lee, B.J., Wijesinghe, W.A., Kim, D., Yang, H., Kim, Y.H., Park, S.J., Jee, Y., Jeon, Y.H.: Radio-protective effect of polysaccharides isolated from *Lactobacillus brevis*-fermented *Ecklonia cava*, *International Journal of Biological Macromolecules*, **2013**, **52**, 260-266;
12. Lee, S.H., Oh, M., Park, J., Jang, S.Y., Cheong, S.H., Lee, H., Moon, S.H.: Antioxidant and anti-inflammatory activities of the ethanolic extract of fermented red ginseng marc, *Food Science and Biotechnology*, **2015**, **24** (4), 651-657;
13. Sun, H., Ni, X., Song, X., Wen, B., Zhou, Y., Zou, F., Yang, M., Peng, Z., Zhu, H., Zeng, Y., Wang, H., Fu, X., Shi, Y., Yin, Z., Pan, K., Jing, B., Zeng, D., Wang, P.: Fermented Yupingfeng polysaccharides enhance immunity by improving the foregut microflora and intestinal barrier in weaning rex rabbits, *Applied Microbiology and Biotechnology*, **2016**, **100** (18), 8105-8120;
14. Sun, H., Ni, X., Zeng, D., Zou, F1., Yang, M., Peng, Z., Zhou, Y., Zeng, Y., Zhu, H., Wang, H., Yin, Z., Pan, K., Jing, B.: Bidirectional immunomodulating activity of fermented polysaccharides from Yupingfeng, *Research in Veterinary Science*, **2017**, **110**, 22-28;
15. Chang, J.S., Chiang, L.C., Hsu, F.F., Lin, C.C.: Chemoprevention against hepatocellular carcinoma of *Cornus officinalis* in vitro, *The American Journal of Chinese Medicine*, **2004**, **32** (05), 717-725;
16. Telang, N.T., Li, G., Sepkovic, D.W., Bradlow, H.L., Wong, G.Y.: Anti-proliferative effects of Chinese herb *Cornus officinalis* in a cell culture model for estrogen receptor-positive clinical breast cancer, *Molecular Medicine Reports*, **2012**, **5** (1), 22-28;
17. Zou, P., Zhao, C., Li, P., Huang, H.: Study on the anti-tumor effect of polysaccharides from *Cornus officinalis* and its immunologic mechanism, *Chinese Journal of Hospital Pharmacy*, **2012**, **32** (1), 20-22;
18. Xu, H.Q., Hao, H.P.: Effects of iridoid total glycoside from *Cornus officinalis* on prevention of glomerular overexpression of transforming growth factor beta 1 and matrixes in an experimental diabetes model, *Biological and Pharmaceutical Bulletin*, **2004**, **27** (7), 1014-1018;
19. Jayaprakasam, B., Olson, L.K., Schutzki, R.E., Tai, M.H., Nair, M.G.: Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (Cornus mas), *Journal of Agricultural and Food Chemistry*, **2006**, **54** (1), 243-248;
20. Cao, G., Cai, H., Cai, B., Tu, S.: Effect of 5-hydroxymethylfurfural derived from processed *Cornus officinalis* on the prevention of high glucose-induced oxidative stress in human umbilical vein endothelial cells and its mechanism, *Food Chemistry*, **2013**, **140** (1-2), 273-279;
21. Ma, W., Wang, K.J., Cheng, C.S., Yan, G.Q., Lu, W.L., Ge, J.F., Cheng, Y.X., Li, N.: Bioactive compounds from *Cornus officinalis* fruits and their effects on diabetic nephropathy, *Journal of Ethnopharmacology*, **2014**, **153** (3), 840-845;
22. Xu, H., Shen, J., Liu, H., Shi, Y., Wei, M.: Morroniside and loganin extracted from *Cornus officinalis* have protective effects on rat mesangial cell proliferation exposed to advanced glycation end products by preventing oxidative stress, *Canadian Journal of Physiology and Pharmacology*, **2006**, **84** (12), 1267-1273;
23. Nawa, Y., Endo, J., Ohta, T.: The inhibitory effect of the components of *Cornus officinalis* on melanogenesis, *Journal of Cosmetic Science*, **2007**, **58** (5), 505-517;
24. Lee, N.H., Seo, C.S., Lee, H., Jung, D.Y., Lee, J.K., Lee, J.A., Song, K.Y., Shin, H., Lee, M.Y., Soe, Y.B., Kim, H., Ha, H.: Hepatoprotective and antioxidative activities of *Cornus officinalis*

against acetaminophen-induced hepatotoxicity in mice, *Evidence-Based Complementary and Alternative Medicine*, **2012**, **2012**, ID 804924;
25. Lee, K.Y., Sung, S.H., Kim, S.H., Jang, Y.P., Oh, T.H., Kim, Y.C.: Cognitive-enhancing activity of loganin isolated from *Cornus officinalis* in scopolamine-induced amnesic mice, *Archives of Pharmacal Research*, **2009**, **32** (5), 677-683;
26. Kam, S.C., Do, J.M., Choi, J.H., Joen, B.T., Roh, G.S., Hyun, J.S.: In vivo and in vitro animal investigation of the effect of a mixture of herbal extracts from *Tribulus terrestris* and *Cornus officinalis* on penile erection, *The Journal of Sexual Medicine*, **2012**, **9** (10), 2544-2551;
27. Park, J.S., Lee, J.S.: The promoting effect of *Cornus officinalis* fermented with *Lactobacillus rhamnosus* on hair growth, *Korean Journal of Pharmacognosy*, **2011**, **42** (3), 260-264;
28. Su, Z., Wang, B.: Preliminary research of fermentation conditions and determination of loganin for *Cornus officinalis* health wine, *Journal of Taiyuan University of Science and Technology*, **2008**, **29** (1), 70-74;
29. Xu, B.T., He, G.Q., Li, X.H., Yu, H.N., Shen, S.R.: Extraction of ursolic acid in *Cornus officinalis* by fermentation combined with ultrasonic-assisted technique, *Journal of Zhejiang University* (Agriculture and Life Sciences), **2009**, **35** (3), 272-277;
30. Liu, J.C., Zhou, R.Q.: Improved mensuration for general flavone of bamboo leaves extract, *Food Science and Technology*, **2005**, **7**, 76-79;
31. Brand-Williams, W., Cuvelier, M.E., Berset, C.: Use of a free radical method to evaluate antioxidant activity, *LWT-Food Science and Technology*, **1995**, **28** (1), 25-30;
32. Hearing, V.J.: Mammalian monophenol monooxygenase (tyrosinase): Purification, properties, and reactions catalyzed, *Methods in Enzymology*, **1987**, **142** (22), 154-165;
33. Lin, C.H., Wei, Y.T., Chou, C.C.: Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi, *Food Microbiology*, **2006**, **23** (7), 628-633;
34. Lin, Y.S., Chen, S.H., Huang, W.J., Chen, C.H., Chien, M.Y., Lin, S.Y., Hou, W.C.: Effects of nicotinic acid derivatives on tyrosinase inhibitory and antioxidant activities, *Food Chemistry*, **2012**, **132** (4), 2074-2080;
35. Choi, H.K., Lim, Y.S., Kim, Y.S., Park, S.Y., Lee, C.H., Hwang, K.W., Kwon, D.Y.: Free-radical-scavenging and tyrosinase-inhibition activities of Cheonggukjang samples fermented for various times, *Food Chemistry*, **2008**, **106** (2), 564-568;
36. Villaño, D., Pecorari, M., Testa, M.F., Raguzzini, A., Stalmach, A., Crozier, A., Tubili, C., Serafini, M.: Unfermented and fermented rooibos teas (*Aspalathus linearis*) increase plasma total antioxidant capacity in healthy humans, *Food Chemistry*, **2010**, **123** (3), 679-683;
37. Stalmach, A., Mullen, W., Pecorari, M., Serafini, M., Crozier, A.: Bioavailability of C-linked dihydrochalcone and flavanone glycosides in humans following ingestion of unfermented and fermented rooibos teas, *Journal of Agricultural and Food Chemistry*, **2009**, **57** (15), 7104-7111;
38. Krafczy, N., Glomb, M. A.: Characterization of phenolic compounds in rooibos tea, *Journal of Agricultural and Food Chemistry*, **2008**, **56** (9), 3368-3376;
39. Peng, L.H., Liu, S., Xu, S.Y., Chen, L., Shan, Y.H., Wei, W., Liang, W.Q., Gao, J.Q.: Inhibitory effects of salidroside and paeonol on tyrosinase activity and melanin synthesis in mouse B16F10 melanoma cells and ultraviolet B-induced pigmentation in guinea pig skin, *Phytomedicine*, **2013**, **20** (12), 1082-1087;
40. Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D.: Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products, *Journal of Agricultural and Food Chemistry*, **1998**, **46** (10), 4113-4117.