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Antibacterial and Antioxidant Effect of Botanical Antimicrobial Agent-Citrus product on Pollack or Ascidian Fishmeat

Jun Ho Jung and Sung Hwan Cho*

Department of Food Science and Technology, Institute of Agriculture and Life Sciences, Gyeongsang National University, Chinju, 660-701, Korea

식물성 천연항균처리에 의한 저장 명태와 우렁쉥이 어육제품의 항균 및 항산화효과

정준호 · 조성환

경상대학교 식품공학과, 농업생명과학연구원

Abstract

The antibiotic and antioxidant effect of botanical antimicrobial agent-citrus product(BAAC) prepared from citrus fruits were investigated by using fishmeats made from pollack and ascidian fillet. We inoculated *Salmonella typhi* into fishmeats that were aferwards treated with BAAC varying the concentration level (50-500ppm) including the control not-treated with BAAC. While specimens were stored at 5℃ for designated period, we measured changes of total number of bacteria, peroxide level and textural properties. In addition, we performed organoleptic examination, focusing on appearance, texture and odor for fishmeats treated with BAAC and stored for designated period. For pollack and ascidian fishmeats, the initial total number of bacteria were 3.7×10^4CFU/g and 7.5×10^4CFU/g, respectively. After they were treated with 250 ppm of BAAC solution and stored for 5 days at 5℃, the figures reduced to 2.5×10CFU/g and 8.4×10CFU/g. No bacteria were found in fishmeats treated with above 500 ppm of BAAC, which remarkably proved antibiotic effect of BAAC solution. Furthermore, all the BAAC-treated specimens exhibited notable antioxidant effect. Fishmeats which were previously treated with 500 ppm of BAAC solution and stored for 30 days at 5℃, showed the lower peroxide level of 16.5 meq/kg than 68.9 meq/kg in the control of pollack and 21.2 meq/kg than 80.7 meq/kg in the control of ascidian. In the results of texture and organoleptic evaluation we could also observe good effects of BAAC on maintaining the freshness of fisher products.

Key words : antibiotic and antioxidant effect, botanical antimicrobial agent-citrus product, peroxide level, organoleptic evaluation, pollack, ascidian, fiahmeat

Introduction

Putrefaction and intoxication due to putrefactive and pathogenic bacterial contamination is one of the serious problems we face today in various fields; from pharmaceutical field to the preservation industry of agricultural, meat and fishery products. Accordingly, disinfectants which have high efficiency against diverse putrescent and pathogenic bacteria meanwhile safe to human physiology have been developed. Unfortunately, up to current date, most of chemical preservatives, which are used for the purpose of restraining multiplication of putrefactive and pathogenic bacteria, show low efficiency within the safety range but show the possibility of intoxication at the level that it is efficient. Taking this fact into consideration, an ideal antibiotic agent must be the one with high efficiency in prevention of bacterial contamination and microbial activity in diverse products or fields, as well as non-toxic and safe to human physiology. In this light, studies are being done with natural botanical antimicrobial agents including grapefruit seed extract(GFSE). GFSE has high antimicrobial efficiency(1-5). and has most of features of a sterilant(6) ; it is antibiotic, tonic, non-toxic(7,8) and enhances antibacterial activity of leukocyte. Botanical antimicrobial agent-citrus mixture(BAAC), which we developed from orange family fruits, is also non-toxic, non-metal, non-corrosive, odorless, and colorless. In addition, its rich content of natural ascorbic acid, tocopherol and polyphenolic compounds prevents

Corresponding author : Sung Hwan, Cho, Department of Food Science and Technology, Institute of Agriculture and Life Sciences, Gyeongsang National University, 900 Kazwa-dong, Chinju, Gyeongnam 660-701, Korea
E-mail : sunghcho@nongae.gsnu.ac.kr

oxidation of agricultural products and keeps original flavor, odor, and color of fishery products. Because BAAC could be expected to reduce deterioration rate of fishery products, we would like report on the results of antibacterial and antioxidant activities of BAAC against fishmeats made from pollack(*Theragra chalcogramma*) and ascidian(*Halocynthia roretzi*) caught along the South.Sea of Korea.

Materials and Methods

Preparation of BAAC

According to the previous method(9), citrus fruits were washed, steeped in the solution of natural antimicrobial agent, and then dried in an infrared ray-equipped dryer through drum-drying process. The dried fruits were weighed and triturated by using Geiger milling system in the good manufacturing practice(GMP). The milled products were fermented in the sealed plastic container, filtered and concentrated to the form of Biomass, to which such biological synergists as natural organic acids and bioflavonoids were added. The mixture was homogenized and standardized to stable liquid product. The final product prepared by GMP, botanical antimicrobial agent-citrus product(BAAC), was used in this experiment on the basis of physicochemical analysis and sensory evaluation for pollack or ascidian fishmeat.

Preparation of fishmeats treated with BAAC

We purchased pollack(*Theragra chalcogramma*) and ascidian(*Halocynthia roretzi*) from the Pusan Public Fish Market and prepared pollack and ascidian fishmeats according to Park's Method(10). After the prepared fishmeats were sterilized in the autovlave they were inoculated with *Salmonella typhi* in this experiment, which is pathogenic and food-borne poisoning, and then treated with BAAC solution in the weight level of 0(Control), 50, 100, 250 and 500 ppm, respectively. The BAAC-treated fishmeats were put into a sterilized polyethylene bag with the control and stored for 5 days or 30 days at 5℃.

Measurement of the number of bacteria

We diluted fishmeats with sterile water using stomach and filtered them. The filtrates were inoculated on the plate count agar and incubated for 48 hours at 30℃. The number of bacteria on the stored fishmeats was counted by paper disk method(9).

Measurement of peroxide value

The antioxidant effect of BAAC was performed for fishmeat specimen prepared according to Filch's method (11). Chloroform-methanol mixture (2 : 1, v/v) was added to the specimen and blended to extract the lipid. The extracted mixtures were kept for 30 days at 5℃. The peroxide level of specimen mixture was measured by the A.O.A.C. method(12) and indicated in meq/kg.

Measurement of textural changes

The condition of Instron texturemeter was represented in the Table 1. From the force-deformation curve, we measured the dimension with planimeter. Hardness is represented as the height of the top point of the first transformation curve, which is necessary to apply pressure up to the set pressure rate(13). Cohesiveness is calculated by the ratio of the dimension of the second transformation curve respect to the dimension of the first transformation curve(14). Elasticity is represented as the distance from the starting point of the second transformation curve to the top point(15). Gumminess is the multiplication of hardness and cohesiveness, and Chewiness is the multiplication of elasticity and gumminess(16).

Table 1. Operation conditions of Instron Texuremeter

Testing instrument	Instron model 1.000
Sample size	2×2×2(㎝)
Ratio of deformation	70%
Cross head speed	50㎜/㎜
Chart speed	50㎜/㎜
Number of bite	2
Area compensation	400
Weight of load cell	5㎏

Sensory evaluation

Ten trained panels performed the organoleptic examination by Hedonic test method(17), regarding color and odor of fishmeats which were treated with BAAC and kept in a polyethylene bag for 5 days at 5℃. The standard of measurement used is as follows:

Dislike extremely	1 point
Dislike very much	2 points
Dislike moderately	3 points
Dislike slightly	4 points
Neither like nor dislike	5 points
Like slightly	6 points
Like moderately	7 points
Like very much	8 points
Like extremely	9 points

The results obtained from sensory evaluation were treated statistically by LSD(Least Significant Difference) test(18).

Results and Discussion

Antibacterial activity of BAAC

The number of *Samonella typhi* according to BAAC is shown in the Table 2. In the specimen not-treated with BAAC, the number of bacteria were individually increased to 49 times of the original number for pollac fishmeat and to 110 times for ascidian fishmeat after 5 days of storage period, whereas the specimen treated with 50 ppm of BAAC showed remarkablly antibacterial effect; the number reduced from 3.7×10^4CFU (colony-forming units)/g to 6.7×10^3CFU/g for pollack fishmeat and from 7.5×10^4CFU/g to 9.5×10^3CFU/g for ascidian fishmeat. In case of 100 ppm, the total number of bacteria in pollack fishmeat reduced to 9.0×10^2CFU/g and to 4.4 x 10^2CFU/g in ascidian fishmeat. Pollack and ascidian fishmeat were completely sterilized with 500 ml of BAAC solution, in which we could achieve 100% antibacterial effect to inhibit the growth of *Samonella typhi*. In Juven's report(19), the experimenter inoculated *Compylobacter jejuni*(10^6CFU/g) to mechanically separated turkey meat, and added 65㎎ of ascorbic acid to each 100g of the meat. As a result of 4 week-storage at 5℃, the number of bacteria was reduced to 10^2CFU/g after 7 weeks. When considered the fact that BAAC includes 3.5% ~ 4.5% of ascorbic acid, it is assumed that the antibacterial effect of BAAC attributes to hydroxyl radical, the cytotoxic substance produced in the reduction process of ascorbic acid(19). BAAC may be expected to reduce the bacterial multiplication under the 500 ppm at low temperature.

Table 2. Effect of BAAC on the antibacterial activity against *Salmonell typhi* inoculated on pollack or ascidian fishmeat treated with BAAC and stored for 5 days at 5℃

(Unit : CFU/g)

Treated concentration (ppm)	Sample	Pollack		Ascidian	
	Storage period (days)	0	5	0	5
0(Control)			1.8×10^6		8.3×10^6
50			6.7×10^3		9.5×10^3
100		3.7×10^4	9.0×10^2	7.5×10^4	4.4×10^2
250			2.5×10		8.4×10
500			0		0

Peroxide value

The changes in peroxide level of the extract from pollack or ascdian fishmeat stored for 30 days at 5℃ are shown in Table 3 or Table 4, respectively. The peroxide level in the untreated specimen increased more rapidly than that treated with BAAC. As in Table 3, peroxide value in the specimen extracted from pollack fishmeat, due to high rancidity rate, radically increased to 41.3meq/㎏ on 10th day, 55.5meq/㎏ on 20th day, and 56.9meq/㎏ on 30th day. On the other hand, all specimens treated with BAAC showed small increase rate up to the 10th day, and as the treated level of BAAC was increased, the increase rate was reduced. For the specimen treated with BAAC at the concentration level of 500 ppm, peroxide value radically exhibited 16.5 meq/kg on the 30th day, which is only 24% of rancidity rate of untreated specimen. In case of ascidian fishmeat in Table 4, the rancidity rate was even higher than that of pollack fishmeat. The peroxide level without any treatment(Control) increased to 67.7meq/㎏ on the 10th day, and to the highest rate on the 20th day. Thereafter, we observed the increase rate of low peroxide value, expecting the decomposition of peroxide compounds; however, it showed radical increase up to 80.7meq/㎏ on the 30th day. The specimen treated with 250 ppm and 500 ppm of BAAC solution showed remarkably antioxidant effect showing 28.0meq/㎏ and 21.2meq/㎏ of low peroxide level on the 30th day. These antioxidant activity of BAAC attributes to the antioxidant components such as tocopherol, ascorbic acid and others. Thus BAAC solution not only seem to prevent the production of toxic substance but also to minimize the destruction of nutrients and food pigments.

Table 3. Changes in peroxide value(meq/kg) of lipids extracted from BAAC-treated pollack fishmeat and stored at 5℃ for 30 days

Treated Concentration of BAAC (ppm)	Storage period (days)			
	0	10	20	30
0(Control)	6.4	41.3	55.5	68.9
50	6.4	19.5	35.7	43.3
100	6.4	18.7	26.6	33.6
250	6.4	15.4	20.9	28.2
500	6.4	11.5	13.4	16.5

Texture After

5 days of storage period at 5℃ the results of textural examination are shown in the Table 5 and Table 6. Table 5

hows textural properties of pollack fishmeat after the storage eriod. Untreated specimen decreased to 54.3% in hardness, 8.5% in elasticity, 51.2% in cohesiveness and 27.7% of the nitial value in chewiness. Nevertheless, with regard to BAAC-treated pollack fishmeats, the textural properties of the pecimen treated with 100 ppm or 500 ppm of BAAC solution howed 81.4% or 87.1% in hardness, 90.2% or 93.5% in lasticity, 72.1% or 83.7% in cohesiveness and 81.4% or 83.7% f the initial value, respectively. We could hence conclude that BAAC is effective in preserving fishmeats. As shown in Table , changes of the textural properties in ascidian fishmeat howed a similar pattern to those of pollack fishmeat. The extural values of the control not-treated with BAAC was lecreased to 35.0% in hardness, 45.1% in elasticity, 41.5% in ohesiveness and 15.8% in chewiness of the initial value after days of storage. The textural properties of ascidian fishmeats reated with 100 ppm or 500 ppm of BAAC solution showed 7.0% or 80.0% in hardness, 60.7% or 65.6% in elasticity, 0.0% or 67.7% in cohesiveness and 75.8% or 87.1% of the nitial value in chewiness, respectively. We could also see that BAAC is effective in preserving ascidian fishmeats. Therefore, he quality of fishery products could be improved through the torage period by treating with BAAC.

Table 4. Changes in peroxide value(meq/kg) of lipids extracted from BAAC-treated ascidian fishmeat and stored at 5℃ for 30 days

Treated Concentration of BAAC (ppm)	Storage period (days)			
	0	10	20	30
0(Control)	12.7	67.7	83.5	80.7
50	12.7	38.2	66.4	64.8
100	12.7	29.0	50.3	55.4
250	12.7	21.1	26.9	28.0
500	12.7	16.9	19.8	21.2

Table 5. Changes in textural property of fishmeat prepared with BAAC-treated pollack fillet* and stored for 5 days at 5℃

Treated Concentration of BAAC (ppm)	Textural properties			
	Hardness	Elasticity	Cohesiveness	Chewiness
0(Control)	3.8	0.63	0.22	0.59
50	4.5	0.78	0.25	1.46
100	5.7	0.83	0.31	1.75
250	5.8	0.88	0.38	1.83
500	6.1	0.86	0.36	1.80

* The intial textural properties showed 7.0 in hardness, 0.92 in elasticity, 0.43 in cohesiveness and 2.15 in chewiness, respectively.

Table 6. Changes in textural property of fishmeat prepared with BAAC-treated ascidian fillet* and stored for 5 days at 5℃

Treated Concentration of BAAC (ppm)	Textural properties			
	Hardness	Elasticity	Cohesiveness	Chewiness
0(Control)	3.5	0.55	0.27	0.49
50	5.5	0.69	0.35	1.76
100	6.7	0.74	0.39	2.35
250	7.5	0.75	0.45	2.66
500	8.0	0.80	0.44	2.70

* The intial textural properties showed 10.0 in hardness, 1.22 in elasticity, 0.65 in cohesiveness and 3.10 in chewiness, respectively.

Organoleptic Examination

Table 7 shows the results of LSD test with organoleptic parameter regarding appearance, odor, texture, and color of fishmeats both untreated(control) and treated with various level of BAAC solution and stored for 5 days at 5℃.

Table 7. Sensory evaluation for pollack or ascidian fishmeat treated with various diluted solution of BAAC, respectively and stored for 5 days at 5℃

Treated Concentration (ppm)	Pollack fishmeat	Ascidian fishmeat
0(Control)	3.4	3.0
50	4.5	5.1
100	6.8	7.2
250	7.9	8.7
500	7.4	8.1

Pollack or ascidian fishmeat treated with 250 ppm of BAAC showed the best scores(7.9 or 8.7) in comparison with 3.4 or 3.0, respectively.

Considering the above-mentioned results, we could conclude the applicability of BAAC on the other fishery products by pursing more sophisticated fundamental studies on BAAC concentration and treatment method to maintain the freshness of fishery products.

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References

1. Cho, S.H., Lee, S.Y., Kim, J.W., Ko, G.H. and Seo, I.W. (1995) Development and application of natural antimicrobial agent isolated from grapefruit seed extract -antimicrobial activities of grapefruit seed extract-. J. Fd Hyg. Safety 10, 33-39
2. Kim, W.Y., Cheong,N.E., Jae, D.Y., Lee, D.C., Kim, J.W., Cho, S.H. and Lee, S.Y. (1994) Characterization of an antimicrobial chitinase purified from the grapefruit extract. Korean J. Plant Pathol., 10, 277-283
3. Lee, T.H., Jeong, S.J., Lee, S.Y., Kim, J.W. and Cho, S.H. (1995) The inhibitory effect of grapefruit seed extracts on the physiological funtionm of *Enterobacter pyrinus*. Korean J. Food Sci. Technol., 27, 985-990
4. Park, W.P., Park, K.D. and Cho, S.H. (1996) Effect of grapefruit seed extract on *Kimchi* fermentation. Foods and Biotechnology 5, 91-93
5. Park, W.P., Lee, D.S. and Cho, S.H. (1999) Effect of grapefruit seed extract and antibrowning agents on the keeping quality of minimally processed vegetables. Acta Hort., 483, 325-330
6. Harich, J. (1982) DF-100. U.S. Patent. 1,354,818, FDA. No. R-0013982.
7. Dunn, G.R. (1981) The acute oral toxicity, skin irritation and corrosivity and acute eye irritation studies of DF-100. project No. 10623 prepared by Bioassay Systems Corperaton.
8. Ko, G. H., Lee, K.H. and Cho, S. H.A (1995) safety test on grapefruit seed extract. J. Korean Soc. Nutr. 24, 690-694
9. Jung, J.H. and Cho, S.H. (2003) Effect of steeping treatment in the natural antimicrobial agent solution on the quality control of processed tofu. Korean Journal of Food Preservation, 10, (Under press)
10. Park, Y. H., Kim, D.S., Cheon, S.K., Kang, J.H. and Park, J.W. (1985) Preparation of fishcakes from red fish meat. Bull. Korean Fish. Soc. 18, 339-351
11. Filch, J., Ascolli, I. Lees, M., Neath, J.A. and Lebaron. F.N. (1957) Preparation of lipid extracts of brain tissues. J. Biol. Chem. 191, 833-841.
12. A.O.A.C. (1980) Official methods of analysis of the association of official analytical chemists. 13th Ed. Washinton, D.C. p.440.
13. Bourne, M.C. (1968) Texture profiles of ripening pears. J. Food Sci., 33, 323-327
14. Kapsalis, J.G., Walker, J.E. and Wolf, M.A (1970) Physicochemical study of the mechanical properties of low and intermediate moisture food. J. Texture Study, 1, 464-468
15. Mohsenin, N.N. (1970) Physical properties of plant an animal materials Vol. 1. Structure, physical characteristics and mechanical properties. Gorden and Breech. Science Pub., N.Y., U.S.A.
16. Breene, W.M. (1975) Application of texture profiles analysis to instrumental food texture evaluation, J. Texture Study, 6, 53-82
17. Cho, S.H., Seo, I.W., Choi, J.D. and Joo, I.S. (1990) Antimicrobial and antioxidant activity of grapefruit seed extract on fishery products. Bull. Korean Fish. Soc. 23, 289-296
18. Kim, B.S., Ahn, Y.K., Yoon, K.J. and Yoon S.U (1987). Statistical Analysis using SPSS. Bakmyung-Sa, Seoul, Korea pp.298-369
19. Juven, B.J., Kanner, J., Weisslowiez, H and Harel, S. (1988) Effect of ascorbic and isoascorbic acids on survival of *Compylobacter jejuni* in poultry meat. J. Food Protection, 51, 436-437.

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