Korean J. Food & Nutr. Vol. 27. No. 2, 164~174 (2014)
http://dx.doi.org/10.9799/ksfan.2014.27.2.164

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Effects of Different Natural Antimicrobial Agents on Marinated Chicken Breast during Storage at Different Temperatures

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저장 온도를 달리하여 저장한 양념 닭가슴살의 천연 항균물질 효과

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국문요약

본 연구는 양념 닭 가슴살의 양념 액에 귤 껍질 추출물(CPE, 2%), 양파 껍질 추출물(OPE, 2%), calcium lactate(2%), 난황 유래 phosvitin(0.1%)과 CPE, OPE 및 calcium lactate 복합처리를 첨가하였을 때 서로 다른 저장온도와 저장기간에 따른 항균 효과 및 품질 변화를 확인하고자 수행되었다. 모든 저장온도와 저장기간에서 CPE, OPE 및 복합 처리군의 총 호기성 세균 수는 대조구에 비해 유의적으로 낮게 확인되었으며, 가장 높은 미생물 생장 억제 효과가 있었던 처리군은 CPE 처리군이었다. 하지만, calcium lactate와 phosvitin 처리군의 미생물의 성장 억제 효과는 나타나지 않았다. pH의 경우, calcium lactate와 OPE 처리군은 모든 저장온도의 저장 0일차에서 대조구에 비해 유의적으로 낮은 값을 나타내었다. OPE 처리군은 모든 저장온도와 저장기간에서 다른 처리구들에 비해 유의적으로 높은 적색도를 나타내었고, CPE와 함께 저장온도에 따른 유의적 차이를 나타내지 않았다. 하지만 이러한 OPE 처리군은 풍미, 맛 및 종합적 기호도에서 다른 처리구들에 비해 유의적으로 낮은 값을 보였으며, calcium lactate와 phosvitin 처리군의 종합적 기호도는 대조구와 유의적 차이가 나타나지 않았다. 결론적으로, 양념 닭가슴살의 CPE, OPE 및 복합처리군에서 저장온도를 달리한 저장기간에 따른 미생물 억제 효과를 확인할 수 있었으나, 관능적 특성을 개선하는 방법의 개발이 필요하다고 판단된다.

Key words: marination, natural antimicrobial agents, chicken breast

Introduction

Marinating chicken breast meat, as a method of value addition, has become an integral part of the chicken meat industry, owing to the increasing demand from consumers, retailers, and the catering sector for processed, ready-to-eat convenience foods (Alvarado & Sams 2004). The important aspects of marination are improvements of microbiological and technological qualities, including flavor, tenderness, water retention, and yield, in addition to extension of shelf life. These improvements benefit both the producers and the consumers (Cannon et al. 1993). Marination by injection is perhaps the most widely used method as it incorporates an exact quantity of brine into the meat by using needles or

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probes, which ensures consistency in the procedure used without the loss of time (Xargayo et al. 2001). Although different formulations are used for marination, the principal ingredients (phosphate, salt, and water) are the same. Meat and poultry marination generally uses sodium acid pyrophosphate, sodium pyrophosphate, sodium tripolyphosphate, or sodium hexametaphosphate, used either alone or in combination (Trout & Schmidt 1983). In terms of acidic marinades, the most common ingredients are organic acid solutions (acetic acid, lactic acid, citric acid, etc.), vinegars, wine, or fruit juices (Burke & Monahan 2003). Antimicrobial and flavoring agents are secondary ingredients added to these formulations in order to improve the shelf life, water holding capacity, and flavor of the meat (Xiong & Kupski 1999). However, consumer concern about the consumption of food formulated with chemical preservatives has increased, and this has created a demand for more natural and minimally processed food. As a result, there has been great interest in natural antimicrobial agents (Cleveland et al. 2001). Furthermore, there are established legislations governing the use of current preservatives to guarantee a high degree of safety (Lis-Balchin & Deans 1997; Hammer et al. 1999).

The emergence of pathogens that are resistant to classical preservatives has also created an urgent necessity to find more effective, alternative antimicrobial agents (Xu & Lee 2001). Citrus flavonoids have a large spectrum of biological activities, including antibacterial, antifungal, antidiabetic, anticancer, and antiviral properties (Burt S 2004; Ortuno et al. 2006). Moreover, flavonoids can function as direct antioxidants and free radical scavengers and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie & Crozier 2000). Specifically, the peel of citrus fruits is a rich source of coumarins, sitosterol, glycosides, and volatile oils (Sultana et al. 2007). Therefore, citrus flavonoids would be effective antimicrobial agents in food systems. Owing to the high antimicrobial activity of onion, it can also be used as a natural preservative to control microbial growth (Pszczola DE 2002). It has previously been shown that fresh onion extract exerts a strong antimicrobial effect because of the presence of both methylcysteine sulfoxide and S-n-propyl cysteine sulfoxide (Corzo-Martinez et al. 2007). Additionally, the flavonoids present in onion peel exhibit a variety of antimicrobial activities. Quercetin and kaempferol are the major dietary flavonoids present in the form of glycosides (Fossen et al. 1998). Recent chemical characterization has shown that sulfur compounds are one of the main active antimicrobial agents (Rose et al. 2005). However, some proteins, namely saponins and phenolic compounds, could also contribute to this activity (Griffiths et al. 2002).

In addition, lactates are approved for use as food additives as they do not possess toxic properties (Aran N 2001). They can be used as adjuvants, nutrient supplements, leavening agents, stabilizers, and thickeners. They are also recognized as safe when used in accordance with good manufacturing practices (Doores S 1990). In recent years, the bacteriostatic and bacteriocidal effects of sodium lactate on some pathogenic bacteria have been established (Weaver & Shelef 1993; Shelef & Potluri 1995). It was also reported that potassium lactate had the ability to delay bacterial growth in low-fat, carrageenan-based patties (Egbert et al. 1992). However, published data on the antimicrobial effect of calcium lactate is limited (Weaver & Shelef 1993; Shelef & Potluri 1995). Furthermore, egg yolk phosvitin is also a good candidate; it contains amphiphilic proteins with chelating ability that kills gram-negative bacteria. This polyanionic phosphoglycoprotein has the ability to bind to multivalent metals such as iron, calcium, and magnesium, and its intensified affinity to lipids is expected to enhance its antimicrobial potential (Khan et al. 2000).

In order to improve the quality characteristics of the final products as well as for consumer satisfaction, factors including marinade ingredients, techniques, processing conditions and equipment are continually being refined and updated. Therefore, the aim of this study was to determine the inhibitory effects of citrus peel extract (CPE), onion peel extract (OPE), calcium lactate, phosvitin, and combinations of these formulations on the growth of microorganisms in marinated chicken breast fillets under an aerobic packaging state at different storage temperatures in order to evaluate their potential use as antimicrobial agents.

Materials and Methods

1. Sample preparation

Fresh skinless chicken breast meat and all necessary ingredients for the production of different marinades were obtained from a local market (Daejeon, Korea). The chicken breast fillets were immediately transported to the laboratory in a polystyrene box containing ice and stored at −18℃ until further use.

2. Marinades

The basal marinade was prepared by mixing corn syrup, sugar, soy sauce, onion, welsh onion, pears, sesame oil, garlic, NaCl, monosodium glutamate, sesame, caramel, and ginger with water. Preliminary trials were conducted to determine the best percentages of CPE, OPE, calcium lactate and phosvitin for microbial inactivation and those values were used in the marinades of the present study. Six treatment marinades were prepared using the basal marinade: i) Control, marinades with ii) 2% (w/w) CPE, iii) 2% (w/w) OPE, iv) 2% (w/w) calcium lactate, v) 0.1% (w/w) phosvitin and vi) combination of CPE, OPE, and calcium lactate. The CPE and OPE were extracted from respective peels for 72 hr at room temperature using 70% (v/v) ethyl alcohol, followed by evaporating the solvent (Kim et al. 2013). Thereafter, the extracts were lyophilized separately using a freeze drier (TFD5505, Il Shin Lab. Co. Ltd., Korea). The combined treatment was prepared using calcium lactate, CPE, and OPE at a ratio of 1:0.5:0.5 (w/w), respectively. The extraction of phosvitin was done according to the method as described by (Ko et al. 2011) and commercially available calcium lactate (ES Food Industry, Korea) was used for this study. All marinades were prepared in the same day of experiment and held at 4℃ until required.

3. Marination and storage of samples

Preliminary trials were conducted to establish the appropriate marinade formulation and cooking conditions to be used in this study. Marination was carried out by injecting the breast meat with the respective marinade at a concentration of 15% (v/w) using a single-needle injector (Doo Won Meditec Co. Ltd., Jollabuk-do, Korea). Marinade was pumped deeply in to the meat piece in every side and allowed homogenous distribution of marinade ingredients throughout the entire piece. Each treated sample was subsequently divided in to small portions (approximately 25 g), aerobically packaged in oxygen-permeable polyethylene bags and stored at 4, 10 and 20℃ until analysis at 0, 3, 6 and 9 days.

4. Microbiological analysis

Microbial analysis was carried out initially after injection of the marinades and at 3, 6 and 9 days of storage at different storage temperatures (4, 10, and 20℃). When the number of total aerobic bacteria counts reached a value higher than 10^7 log CFU/g, microbial analysis and other determinations were terminated. Each sample (5 g) was cut into small pieces and homogenized for 2 min in a sterile stomacher bag (bag mixer400; Interscience Co., St. Nom la Breteche, France) containing 45 ㎖ of sterile saline (0.85%, w/v). Then, those were serially diluted in sterile saline (0.85%), and each diluent (0.1 ㎖) was spread on respective bacterial media. Plate count agar and eosine methylene blue agar (Difco Laboratories, NJ, USA) were used for total bacterial flora and coliforms, respectively. The plates were incubated at 37℃ for 48 hr, and microbial counts were expressed as log CFU/g.

5. pH and instrumental color measurement

Each sample (1 g) was homogenized with 9 ㎖ of distilled water using a mechanical homogenizer (IKA Laboratory Equipment, Seoul, Korea) and filtered (Whatman No. 4, GE Healthcare UK Limited, Buckinghamshire, UK). The pH value of the filtrate was measured using an electronic pH meter (SevenGo, Mettler-Toledo Inti, Inc, Schwerzenbach, Switzerland). The surface color measurements (CIE L*, a*, and b* values representing lightness, redness and yellowness, respectively) of the marinated chicken breast meat was evaluated immediately after the sampling and during the storage period using a colorimeter (Spectrophotometer, CR-300, Minolta Inc., Tokyo, Japan) which was calibrated against a black and a white reference tile. Measurement of L*, a* and b* values were taken at three different locations of each sample as a observation number.

6. Sensory evaluation

In the present study, the marinated chicken samples were evaluated for sensory qualities at the first day of sampling. The samples (2.0×3.0×1.5 ㎝) were pan-fried for 4 min to achieve a core temperature of approximately 72℃ as measured by a digital thermometer (YF-160A-type-K, YFE, Taiwan). Water was provided between samples to cleanse the oral cavity. Each sample was placed in a white plastic tray with randomly coded with 3 digit number and provided for evaluation. The cooked samples were evaluated for color, odor, flavor, taste, tenderness and overall acceptability by seven semi-trained panelists who have experience in sensory evaluation of chicken meat more than 1 year. A 9-point hedonic scale was used (9=like extremely, 5=like moderately 1=dislike extremely) in this study.

7. Statistical analysis

The whole experimental procedures were triplicated. Statistical analysis was performed using one-way analysis of variance by the procedure of General Linear Model using SAS program version 9.1 (SAS, 2004, SAS Institute, Cary, NC, USA). The differences among the mean values were identified using Duncan's multiple range tests at a confidence level of $p<0.05$. Mean values and standard errors of the means are reported.

Results and Discussion

1. Microbiological properties

The changes in the microbial population (log CFU/g) of the marinated chicken breast meat during storage at different temperatures are shown in Table 1. The microbial population was significantly affected by the different treatments, storage times, and storage temperatures. The addition of CPE, OPE, or a combination of these to the marinades resulted in a significant reduction ($p<0.05$) of the total aerobic bacterial

Table 1. Total aerobic bacterial number (log CFU/g) of the marinated chicken breast meat after addition of natural extracts during storage at different temperatures

Storage temperature	Treatment[1)]	Storage period (days)				SEM[2)]
		0	3	6	9	
4℃	Control	3.54^{az}	5.57^{ay}	7.48^{bx}	8.19^{aw}	0.040
	Citrus peel extract	3.24^{dz}	5.25^{by}	7.08^{ex}	7.16^{dw}	0.017
	Calcium lactate	3.56^{az}	5.53^{ay}	7.54^{ax}	8.20^{aw}	0.034
	Onion peel extract	3.40^{cz}	5.14^{by}	7.36^{cx}	7.63^{bw}	0.034
	Phosvitin	3.56^{az}	5.56^{ay}	7.48^{bx}	8.13^{aw}	0.016
	Combination	3.49^{bz}	5.22^{by}	7.28^{dx}	7.49^{cw}	0.026
	SEM[3)]	0.013	0.038	0.015	0.039	
10℃	Control	3.54^{az}	6.91^{by}	8.85^{bx}	-[4)]	0.018
	Citrus peel extract	3.24^{dz}	6.06^{ey}	7.47^{fx}	-	0.024
	Calcium lactate	3.56^{az}	6.93^{by}	8.98^{ax}	-	0.025
	Onion peel extract	3.40^{cz}	6.57^{cy}	8.16^{dx}	-	0.022
	Phosvitin	3.56^{az}	7.10^{ay}	8.48^{cx}	-	0.018
	Combination	3.49^{bz}	6.40^{dy}	8.04^{ex}	-	0.023
	SEM[3)]	0.013	0.019	0.029		
20℃	Control	3.54^{ay}	8.58^{ax}	-	-	0.018
	Citrus peel extract	3.24^{dy}	7.81^{dx}	-	-	0.035
	Calcium lactate	3.56^{ay}	8.27^{bx}	-	-	0.012
	Onion peel extract	3.40^{cy}	8.13^{cx}	-	-	0.022
	Phosvitin	3.56^{ay}	8.30^{bx}	-	-	0.012
	Combination	3.49^{by}	8.05^{cx}	-	-	0.015
	SEM[3)]	0.013	0.026			

[1)] Control (basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5: 0.5: 1.0% [w/w]).

[2)] Standard error of the mean (n=12), [3)] (n=18).

[4)] Experiment was not performed because microbial population was over 7 log CFU/g.

[a~e] Indicates statistical significance within column ($p<0.05$). [w~z] Indicates statistical significance within row ($p<0.05$).

counts in the finished product compared to the other treatments. The mean decrease in microbial population in the samples treated with CPE; OPE; and in combination was 0.3, 0.14, and 0.05 log CFU/g, respectively. Coliform bacteria were not detected in any of the samples (data not shown). The subsequent storage of chicken samples resulted in a rapid increase in total aerobic bacterial counts to values greater than 7 log CFU/g in control samples and those treated with calcium lactate and phosvitin compared to other treatments. A clear increase in the microbial population, of greater than 7 log CFU/g, was observed on day 6 with the remaining treatments, when stored at 4℃. CPE treatment had the lowest microbial count on that day. The highest inhibitory effect, during storage at 4℃, was found in the samples treated with CPE followed by those treated with a combination of different agents and OPE.

During storage at 10℃, CPE, OPE, and a combination of the two exerted similar effects in terms of microbial inhibition ability. Generally, the effects of calcium lactate and phosvitin on microbial inhibition were lower than those of the other treatments. Microbial populations greater than 7 log CFU/g were observed on day 6 of storage for all treatments at 10℃, and the microbial populations at 10℃ were higher than that at 4℃. Therefore, the shelf life is limited to less than 3 days at 10℃ compared to around 6 days at 4℃. In contrast, our results demonstrate that storage at 20℃ represented the lowest shelf life period at less than 1 or 2 days. Similar to the aforementioned two storage temperatures, at 20℃ a significantly higher reduction was observed with CPE followed by OPE treatments compared to the others.

This inhibitory effect of CPE could be attributed to the fact that citrus contains various kinds of flavonoids, including hesperidin, naringin, nobiletin, anthocyanins, and coumarin, which are known to have inhibitory effects on microbial growth (Elisa et al. 2013). The *in vitro* studies of Yi et al. (2008) showed on a broad antimicrobial spectrum of citrus extracts against both gram-negative and gram-positive bacteria where hesperidin played a major role in inhibition. Our previous study found that CPE and OPE had major inhibitory effects on microbial growth in seasoned chicken meat (Alahakoon et al. 2013). The results of the present study agree with previous findings on the inhibitory effects of citrus against *Salmonella* Typhimurium, *Escherichia coli*, and *Staphylococcus aureus* (Javed et al. 2011). Our results are in complete agreement with those previously reported (Rauha et al. 2000; Rose et al. 2005). Antimicrobial properties were observed after the addition of onion powder to pork loin and belly, where it reduced the total plate counts and Enterobacteriaceae (Park et al. 2008). Moreover, Zohri et al. (1995) found that onion oil was highly active against different species of gram-positive and gram-negative bacteria. In addition, many flavonoids found in onion, such as quercetin, kaempferol, anthocyanin, and high organic sulfur compounds have been reported to possess antibacterial and antifungal properties (Rauha et al. 2000).

2. Physical properties

As shown in Table 2, the addition of calcium lactate and OPE exhibited a greater influence on pH reduction during the start of the storage period compared to other treatments. In addition, OPE, and its combination treatment, could maintain relative consistency in pH during the storage period, unlike other treatments. In the samples stored at 4℃, CPE, OPE, and their combined treatments maintained pH at 5.96, 5.45, and 6.02, respectively, on day 6, whereas all the other treatments showed an increase in pH. Aerobic bacteria grew rapidly in these samples and reached a count of 7 log CFU/g after 6 days of storage at 10℃, which resulted in deamination of amino acids. The highest pH values throughout the storage period and at all storage temperatures were recorded in control samples followed by phosvitin-treated samples. The increased pH observed during the storage period after treatment with calcium lactate and phosvitin could be due to proteolysis and, as Bianchi et al. (2009) reported, it could be due to the alkaline properties of marinades, which result in a change in the cellular buffer.

Tables 3, 4, and 5 show the Hunter color values of marinated chicken meat stored at 4℃, 10℃, and 20℃, respectively. An increase in redness (a* values) was observed in samples treated with calcium lactate and a combination of CPE, OPE, and calcium lactate, whereas a gradual declining trend was observed in the phosvitin-treated samples stored at 4℃. Yellowness and lightness were relatively stable in phosvitin-treated samples when compared to the controls. These results agree with a previous study that found a decrease in redness and stability in yellowness and lightness over time in phosvitin-treated ground beef samples (Jung et al. 2013). However, the reduction in the red color

Table 2. pH changes of the marinated chicken breast after the addition of different natural extracts during storage at different temperatures

Storage temperature	Treatment[1)]	Storage period (days)				SEM[2)]
		0	3	6	9	
4℃	Control	6.13[abx]	6.19[bz]	6.53[by]	6.99[ax]	0.038
	Citrus peel extract	6.09[abx]	5.81[dz]	5.96[cy]	6.68[aw]	0.040
	Calcium lactate	5.97[bx]	5.96[cz]	6.29[by]	6.74[ax]	0.054
	Onion peel extract	5.95[bx]	5.83[dxy]	5.45[dy]	5.65[bxy]	0.120
	Phosvitin	6.17[ax]	6.36[ay]	6.85[ax]	5.94[bz]	0.090
	Combination	6.07[abw]	5.94[c]	6.02[c]	5.94[b]	0.079
	SEM[3)]	0.041	0.023	0.084	0.118	
10℃	Control	6.13[aby]	6.13[by]	6.95[ax]	-[4)]	0.029
	Citrus peel extract	6.09[abx]	5.21[dy]	6.09[bx]	-	0.066
	Calcium lactate	5.97[by]	5.87[cy]	6.66[ax]	-	0.068
	Onion peel extract	5.95[bx]	5.67[cxy]	5.45[cy]	-	0.095
	Phosvitin	6.17[ay]	6.67[ax]	6.82[ax]	-	0.109
	Combination	6.07[abx]	5.67[cy]	6.05[bx]	-	0.036
	SEM[3)]	0.041	0.063	0.102		
20℃	Control	6.13[aby]	6.44[bx]	-*	-	0.015
	Citrus peel extract	6.09[ab]	6.00[bc]	-	-	0.104
	Calcium lactate	5.97[b]	5.84[c]	-	-	0.122
	Onion peel extract	5.95[b]	5.95[bc]	-	-	0.167
	Phosvitin	6.17[ay]	7.02[ax]	-	-	0.098
	Combination	6.07[abx]	5.55[cy]	-	-	0.014
	SEM[3)]	0.041	0.052			

[1)] Control (basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5: 0.5: 1.0% [w/w]).
[2)] Standard errors of the means (n=12), [3)] (n=18).
[4)] Experiment was not performed because microbial population was over 7 log CFU/g.
[a~e] Indicates statistical significance within column (p<0.05). [x,y] Indicates statistical significance within row (p<0.05).

intensity of phosvitin-treated samples during storage could be attributed to the interdependence between lipid oxidation and color oxidation in meats. Discoloration of meat products results from oxidation of oxymyoglobin to metmyoglobin, and the process is further accelerated by lipid oxidation and/or modification of the heme molecular structure during protein oxidation (Estévez & Cava 2004; Rojas & Brewer 2008). However, the iron-binding properties of phosvitin could decrease the lipid and protein oxidation caused by the available free irons (Extévez & Cava 2004).

In addition, OPE and CPE caused no significant changes in a* value of the samples at any storage temperature. However, there was a significant difference in the lightness value between CPE-treated and other samples on the first day of treatment. Subsequent storage resulted in an increase in lightness value at all storage temperatures in the above-mentioned samples. This is in agreement with a previous study where lightness increased when albedo was added to bologna sausages (Fernandez-Lopez et al. 2004). According to the findings of Hanan et al. (2013), lightness significantly increased in ground beef compared to controls during 21 days of refrigerated storage. In addition, lightness increased when citrus fiber and spice essential oils were added to bologna sausages. This increase is likely due to the fact that fiber is structurally composed of macromolecules that are rehydrated and present outside the meat matrix, thus affecting

Table 3. Hunter color values of injection-marinated chicken breast after the addition of different natural extracts during storage at 4℃

Treatment[1)]	L* (day)				SEM[2)]	a* (day)				SEM[2)]	b* (day)				SEM[2)]
	0	3	6	9		0	3	6	9		0	3	6	9	
Control	56.46^{x}	56.68^{xa}	54.21^{cxy}	51.06^{cy}	1.265	3.21^{bcy}	4.71^{bxy}	4.83^{bx}	4.28^{bxy}	0.460	12.15^{b}	14.92^{d}	13.57^{c}	14.22^{c}	1.421
Citrus peel extract	55.32^{y}	56.23^{ay}	62.86^{ax}	63.53^{ax}	1.274	5.25^{b}	4.46^{b}	4.17^{b}	4.78^{b}	0.585	28.33^{a}	30.94^{a}	30.96^{a}	31.06^{a}	2.232
Calcium lactate	58.14	56.72^{a}	59.24^{b}	56.65^{b}	0.915	1.99^{cy}	3.56^{b}	4.26^{b}	3.88^{b}	0.665	14.85^{bx}	15.01^{d}	14.64^{c}	13.62^{c}	0.794
Onion peel extract	52.79^{x}	48.56^{by}	48.80^{dy}	53.86^{cx}	0.871	9.62^{a}	10.26^{a}	9.52^{a}	8.85^{a}	0.797	24.45^{ax}	22.16^{bxy}	21.84^{bxy}	19.96^{by}	0.930
Phosvitin	55.81	56.20^{a}	57.27^{b}	53.65^{bc}	1.616	3.94^{bcx}	2.68^{b}	2.82^{b}	3.26^{b}	0.821	13.05^{bxy}	12.07^{d}	14.14^{c}	13.11^{c}	0.942
Combination	52.88	51.11^{a}	50.93^{d}	53.35^{bc}	0.763	6.03^{by}	4.90^{bz}	7.53^{axy}	8.08^{ax}	0.484	21.60^{a}	19.57^{cyz}	19.05^{bz}	23.20^{bx}	0.710
SEM[3)]	1.289	1.400	0.872	0.980		0.680	0.522	0.792	0.574		1.856	0.801	0.942	1.272	

[1)] Control (basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5:0.5:1.0% [w/w]).

[2)] Standard errors of the means (n=12), [3)] (n=18).

[a~e] Indicates statistical significance within column (p<0.05). [x,y] Indicates statistical significance within row (p<0.05).

Table 4. Hunter color values of injected chicken breast after the addition of different natural extracts during storage at 10℃

Treatment[1)]	L* (day)				SEM[2)]	a* (day)				SEM[2)]	b* (day)				SEM[2)]
	0	3	6	9		0	3	6	9		0	3	6	9	
Control	56.46	56.35^{b}	52.68^{b}	-[4)]	1.403	3.21^{bcy}	3.44^{b}	4.58^{b}	-	0.637	12.15^{by}	13.05^{dxy}	15.81^{cx}	-	0.944
Citrus peel extract	55.32^{y}	61.28^{ax}	63.60^{ax}	-	1.041	5.25^{b}	4.16^{b}	3.85^{b}	-	0.622	28.33^{a}	27.03^{a}	28.49^{a}	-	2.316
Calcium lactate	58.14	57.17^{b}	57.27^{ab}	-	1.271	1.99^{cy}	5.37^{bx}	3.00^{bxy}	-	0.751	14.85^{bx}	13.30^{dy}	13.72^{cxy}	-	0.390
Onion peel extract	52.79^{y}	52.07^{by}	57.86^{abx}	-	1.132	9.62^{a}	9.86^{a}	7.68^{a}	-	0.749	24.45^{ax}	19.51^{cy}	21.65^{bxy}	-	0.895
Phosvitin	55.81	55.33^{b}	57.31^{ab}	-	1.900	3.94^{bcx}	3.51^{b}	3.17^{b}	-	0.679	13.05^{b}	13.12^{d}	13.30^{c}	-	0.809
Combination	52.88	52.01^{b}	52.93^{b}	-	1.087	6.03^{b}	6.71^{b}	7.48^{a}	-	0.730	21.60^{a}	22.24^{b}	22.36^{b}	-	1.501
SEM[3)]	1.289	1.193	1.511			0.680	0.798	0.596			1.856	0.730	1.039		

[1)] Control (basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5:0.5:1.0% [w/w]).

[2)] Standard error of the mean (n=9), [3)] (n=18).

[4)] Experiment was not performed because microbial population was over 7 log CFU/g.

[a~e] Indicates statistical significance within column (p<0.05). [x,y] Indicates statistical significance within row (p<0.05).

Table 5. Hunter color values of injected chicken breast after the addition of different natural extracts during storage at 20℃

Treatment[1)]	L* (day)				SEM[2)]	a* (day)				SEM[2)]	b* (day)				SEM[2)]
	0	3	6	9		0	3	6	9		0	3	6	9	
Control	56.46	55.20^{bc}	-[4)]	-	1.290	3.21^{bc}	2.07^{c}	-	-	0.342	12.15^{b}	12.88^{c}	-	-	1.129
Citrus peel extract	55.32^{y}	65.40^{ax}	-	-	1.297	5.25^{b}	3.06^{bc}	-	-	0.671	28.33^{a}	26.94^{a}	-	-	2.950
Calcium lactate	58.14	59.23^{b}	-	-	1.067	1.99^{c}	4.16^{bc}	-	-	0.928	14.85^{b}	13.57^{c}	-	-	0.776
Onion peel extract	52.79	52.79^{bc}	-	-	0.952	9.62^{a}	8.83^{a}	-	-	0.727	24.45^{a}	21.73^{b}	-	-	1.389
Phosvitin	55.81	51.35^{c}	-	-	1.478	3.94^{bc}	3.97^{bc}	-	-	0.596	13.05^{b}	13.61^{c}	-	-	1.032
Combination	52.88	57.83^{b}	-	-	1.060	6.03^{b}	5.47^{b}	-	-	0.579	21.60^{a}	21.35^{b}	-	-	1.337
SEM[3)]	1.289	1.113				0.680	0.594				1.856	1.294			

[1)] Control (basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5:0.5:1.0% [w/w]).

[2)] Standard error of the mean (n=6), [3)] (n=18).

[4)] Experiment was not performed because microbial population was over 7 log CFU/g.

[a~e] Indicates statistical significance within column (p<0.05). [x,y] Indicates statistical significance within row (p<0.05).

color coordinates such as lightness (Viuda-Martos et al. 2010). Redness of CPE-treated samples stored at all temperatures did not change with storage time, and it was lower only than that of OPE-treated samples. However, citrus peel contains major pigment carotenoids, which impart a yellow color, while cryptoxanthin and β-citraurin impart the orange and red colors. The a* values that were enhanced with OPE treatment were greater (p<0.05) than those enhanced with the other treatments at all storage temperatures throughout the storage period whereas the change in lightness was the lowest. This red color did not deteriorate during the entire storage period. Potential antioxidants found in onions may inhibit the color oxidation of chicken meat samples, thus producing the red color and maintaining it for the duration of the storage period. The decreased lightness caused by the surface darkening of meat with onion juices may be due to the increased binding reaction of myoglobin and myofibrillar proteins (Kim et al. 2009). Furthermore, b* values of CPE-treated chicken samples were significantly greater (p<0.05) than those of the other treatments.

3. Sensory characteristics

Higher values of overall acceptability were recorded for the control, calcium lactate-, and phosvitin-treated samples, whereas OPE-treated samples had the lowest score (Table 6). Moreover, a similar acceptability was obtained for samples with CPE or combined treatment with the exception of color and odor values. These values were higher for samples that were subjected to the combined treatment than for the CPE-treated samples. Furthermore, it was found that OPE-treated samples had the lowest scores for flavor and taste compared to other treatments, whereas calcium lactate- and phosvitin-treated samples had significantly higher scores. In addition, OPE- and calcium lactate-treated samples exhibited the lowest tenderness scores compared to the other treatments. The pH of meat injected with marinade containing OPE was lower than those of other treatments in the present study, which may result in protein denaturation causing decreased tenderness as shown by Yusop et al. (2010). However, our observations of decreased tenderness of calcium lactate-treated samples were not consistent with those of with previous studies. Calcium likely acts as a postmortem activator of calpain enzymes in decreasing shear force (Koohmaraie M 1994). In addition, Lawrence et al. (2003a, 2003b) pointed out that injection enhancement with several calcium solutions increased the sensory scores for tenderness and juiciness. However, the sensory panel used in this study could detect improvements in tenderness after the addition of CPE, phosvitin, and a combination of natural extracts.

In addition, the combined treatment had a considerably higher acceptance in terms of color, odor, and tenderness, whereas flavor and taste were negatively affected by the same treatment. The aroma and flavor of onion in marinated chicken meat remained unchanged even after cooking. Onion produces sulfuric odor and flavor, but those are not considered as off-odor or off-flavors. However, too strong an onion odor and flavor could be offensive to some consumers (Rico et al. 2007). It has also been reported that higher

Table 6. Sensory scores of marinated chicken breast after the addition of different natural extracts

Treatment[1)]	Sensory parameter					
	Color	Odor	Flavor	Taste	Tenderness	Overall acceptability
Control	5.08^{ab}	5.08^{ab}	5.04^{a}	5.08^{a}	5.29^{a}	5.16^{a}
Citrus peel extract	4.75^{b}	4.58^{b}	4.08^{b}	3.79^{b}	5.33^{bc}	4.04^{b}
Onion peel extract	4.71^{b}	5.04^{ab}	3.08^{c}	2.46^{c}	4.42^{b}	2.50^{c}
Calcium lactate	5.08^{ab}	5.21^{ab}	5.00^{a}	4.79^{a}	4.79^{ab}	4.88^{a}
Phosvitin	5.21^{ab}	5.25^{ab}	5.00^{a}	5.04^{a}	5.33^{a}	5.29^{a}
Combination	5.42^{a}	5.46^{a}	3.79^{b}	3.54^{b}	5.21^{a}	3.63^{b}
SEM[2)]	0.211	0.227	0.202	0.203	0.260	0.196

[1)] Control (Basal marinade), control + 2% (w/w) citrus peel extract, control + 2% (w/w) calcium lactate, control + 2% (w/w) onion peel extract, control + 0.1% (w/w) phosvitin, control + combination of citrus peel extract, onion peel extract, and calcium lactate (0.5:0.5:1.0% [w/w]).

[2)] Standard error of the mean (n=42). $^{a-c}$ Indicates statistical significance within column (p<0.05).

concentrations (0.5%) of onion resulted in a reduced sensory quality in the chicken meat after cooking (Yang et al. 2011). The characteristic flavor and aroma of plants in the onion family Alliaceae result from the enzymatic hydrolysis of the S-alkenyl-l-cysteine sulfoxides producing volatile sulfur compounds and by-products, including pyruvic acid and ammonia (Randle et al. 1995). These compounds caused the strong onion odor and flavor. Moreover, the lower flavor score with orange peel is probably due to its higher content of acetic acid, which negatively affects the flavor (Aleson-Carbonell et al. 2003). In agreement with our current findings, Mexis et al. (2012) showed that the samples containing citrus extract conveyed a fruity flavor, which is a negative addition to the typical chicken flavor.

Conclusion

CPE, OPE, and their combination with calcium lactate were the most effective in the prevention of total aerobic bacteria growth during storage. Therefore, these marinades could be used as natural preservations to extend the shelf life of chicken breast. Furthermore, these marinades resulted in enhanced stability of meat color over time. However, the sensory attributes of CPE and OPE marinades may be objectionable and the use of effective methods or modified formulations to overcome this, while maintaining the desirable effects, may be required.

Acknowledgements

This research was supported by Golden Seed Project, Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development of Administration (RDA) and Korea Forest Services (KFS).

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접　　수 : 2013년 11월 26일
최종수정 : 2014년 3월 25일
채　　택 : 2014년 3월 29일