EFFECTS OF ELECTRON BEAM IRRADIATION AND HIGH PRESSURE TREATMENT COMBINED WITH CITRUS PEEL EXTRACT ON SEASONED CHICKEN BREAST MEAT

AMALI U. ALAHAKOON¹, DINESH D. JAYASENA^{1,2}, SAMOOEL JUNG¹, SUN HYO KIM¹, HYUN JOO KIM³ and CHEORUN JO^{3,4}

¹Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea

²Department of Animal Science, Uva Wellassa University, Badulla, Sri Lanka

³Department of Agricultural Biotechnology, Center for Food and Bioconvergence) and Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea

⁴Corresponding author: TEL: +8228804804; FAX: +8228732271; EMAIL: cheorun@snu.ac.kr

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ABSTRACT

This study compared the effects of electron beam irradiation (EB; 1 and 2 kGy) and high pressure processing (HP; 300 and 400 MPa) on the quality characteristics of marinated chicken breast meat with and without citrus peel extract (CPE; 2%). The combination of CPE with EB (2 kGy) had a significant antimicrobial effect among the treatments. EB alone at 2 kGy reduced the initial microbial counts by 3.16 log cfu/g and no viable cells were detected in samples with CPE. The addition of CPE was not effective to control lipid oxidation in the seasoned breast meat during refrigerated storage. Both EB and HP had a nonsignificant impact (P > 0.05) on sensory attributes of samples without CPE. The use of CPE followed by EB (2 kGy) was the most effective way for shelf life extension of marinated chicken breast but adversely affected the sensory characteristics.

PRACTICAL APPLICATIONS

Nonthermal technologies, such as electron beam irradiation and high pressure processing, have a potential to be used in the meat industry as hurdle technology together with antimicrobial agents. Citrus peel extract can be an interesting antimicrobial alternative to be used together with nonthermal technologies as it is an effective antimicrobial agent responsible for the shelf life extension of meat systems. Furthermore, this could be a better approach to reduce the intensity of these technologies, which ensures only minor effects on sensory and nutritional qualities. Currently, the consumer demand for seasoned chicken breast products is increasing dramatically throughout the world. However, shelf life improvement of such products has become a critical issue for meat processors. Thus, the combination of these cost-effective technologies will enhance the shelf life of marinated products without affecting the sensory attributes of products.

INTRODUCTION

Over the past decades, meat has been marinated to improve flavor, tenderness and product shelf life. The most commonly used marinades include salt and sodium tripolyphosphate in addition to secondary ingredients such as antimicrobial and flavoring agents to ensure product quality and safety (Alvarado and McKee 2007). However, Bjorkroth (2005) reported that there were higher bacterial levels in the marinated products compared with nonmarinated products and microbial stability of poultry meat has not been enhanced by marination alone.

Marinades often contain natural additives such as herbs and spices as flavoring ingredients. These additives are known to contain complex mixtures of active components (Angioni *et al.* 2004). It is of interest to determine the possibility of these natural additives to act as antimicrobial agents to enhance the shelf life of marinated products as there is a growing consumer demand toward natural additives (Jayasena and Jo 2013).

Citrus peel is a natural source of antimicrobial and antioxidant additives such as naringin, hesperidin, neohesperidin, rutin and naringenin (Kawaii *et al.* 1999; Gil-Izquierdo *et al.* 2002). Citrus peel contains numerous biologically active compounds, including phenolic acids and flavonoids, which have a large spectrum of activities: antibacterial, antifungal, antidiabetic, anticarcinogenic and antiviral properties (Ortuno *et al.* 2006). Besides, previous studies have revealed that citrus extract is an effective antimicrobial agent responsible for the shelf life extension of meat systems (Yi *et al.* 2008; Alahakoon *et al.* 2013).

The use of multiple hurdles involves the combination of different antimicrobial agents or some antimicrobial agents with a nonthermal treatment or moderate thermal treatment. Different hurdles will prevent multiplication of microorganisms, inactivate them or cause them to die (Corbo *et al.* 2009). The nonthermal preservation techniques, including high pressure processing (HP), high-intensity pulsed light, X-rays and electron beam irradiation (EB), have a potential to be used in the meat industry in hurdle technology together with antimicrobial agents (Dincer and Baysal 2004). Nonthermal preservation can act as an initial hurdle in reducing the spoilage and pathogenic microflora, whereas the antimicrobial agents are effective hurdles during the storage time as they inhibit the growth of surviving cells (Hugas *et al.* 2002).

Garcia-Graells *et al.* (1999) and Rastogi *et al.* (2007) stated that the use of HP, combined with bacteriocins and natural antimicrobials, could result in a longer shelf life than HP or antimicrobial agents alone could. The aforementioned synergistic effect has been attributed to the sublethal injury occurred in the bacterial population, which facilitates the access of antimicrobial compounds to the cellular targets (Espina *et al.* 2010). As the synergistic combinations provide an opportunity to decrease the intensity of the each hurdle used for food preservation, nutritional and sensorial properties of food can be maintained (Leistner and Gorris 1995; Kim *et al.* 2014).

Irradiation has several applications in the food industry to improve safety, shelf life and quality of food compared with heat-processed foods and to maintain nutritional value. Currently, several countries have permitted food irradiation and more than half a million tons of food are irradiated annually (Eustice and Bruhn 2013). Moreover, the use of irradiation in meat is restricted to raw and packaged poultry at 1.5 and 3 kGy, respectively, while maximum dose for fresh and frozen red meat are 4.5 and 7 kGy, respectively (Sommers 2004). Irradiation is an effective means to eliminate pathogens, although it has a possibility to alter the meat quality depending on the dose (Du *et al.* 2002; Kang *et al.* 2012). Therefore, low-dose irradiation is practiced in the meat industry to minimize these alterations (Clardy *et al.* 2002). However, the key issue regarding the low-dose irradiation is the survival of certain pathogens (Clardy *et al.* 2002). Consequently, additional hurdles are necessary to control bacterial growth during storage of irradiated meat and meat products. On the contrary, the use of HP in food processing steadily increased over the past several years to enhance food safety by inactivating microorganisms (Garriga and Aymerich 2009). In particular, the variety of HP-treated meat products have risen dramatically, and cured ham and some precooked meals containing poultry, pork, chorizo and various types of sausages are now available on the market (Garriga and Aymerich 2009).

Therefore, this study was designed to determine the combined effect of citrus peel extract (CPE) with either EB or HP on the microbial safety and sensory properties of chicken breast meat during refrigerated storage.

MATERIALS AND METHODS

Sample Preparation

The fresh skinless chicken breast meat and all necessary ingredients for the production of marinades were obtained from a local market (Daejeon, Korea). The chicken breast meat samples were immediately transported to the laboratory in a polystyrene box containing ice and stored at -18C until further use. CPE was prepared by treating citrus peels with 70% ethyl alcohol (1:3, w/v) for 72 h at room temperature (approximately 20C) and evaporating the solvent (Kim *et al.* 2013). Thereafter, the extract was lyophilized using a freeze drier (TFD5505, Il Shin Lab. Co., Ltd., Seoul, Korea).

The marinade solution was prepared using water, salt, sugar, sodium pyrophosphate and monosodium glutamate with or without CPE (2% w/v). The marinade was mixed thoroughly with the chicken breast meat (approximately 25 g) by hand massaging for 5 min to enhance maximum absorption. Each sample was vacuum packed and then subjected to EB or HP treatments.

EB and HP Treatments

For EB treatment, the samples were irradiated on both sides by a linear electron beam accelerator (energy, 2.5 MeV; beam power, 40 kW; EB Tech., Daejeon, Korea). The beam current was 0–4.5 mA. Irradiation was performed at a conveyor velocity of 10 m/min and a dose rate of 1.1–2.2 kGy/s. Alanine dosimeters, attached to the top and bottom surfaces of the sample packs, were read using a 104 Electron Paramagnetic Resonance unit (Bruker Instruments, Inc., Bullerica, MA) to confirm the target doses, which were 0, 1 and 2 kGy in this study.

The HP treatment of samples (approximately 25 g) was carried out at Korea Food Research Institute (Seongnam,

Korea). The samples were placed in a pressure vessel submerged in a hydrostatic fluid medium (Quintus food processor 6, ABB Autoclave Systems, Inc., Columbus, OH) and subjected to HP treatment at 300 and 400 MPa for 5 min, with the initial temperature of the pressure vessel at $15 \pm 3C$. The hydrostatic fluid was a mixture of deionized water and water glycol-type fire-resistant hydraulic fluid (Houghto-safe 620-TY, Houghton International, Inc., Valley Forge, PA). The rate of pressurization was 5–7 MPa/s and pressure in the chamber was released within 10 s. The samples were stored at 4C until further analyses up to 9 days at 3-day intervals.

Microbiological Analysis

Microbial analysis of seasoned meat samples was carried out initially after EB or HP treatment and then at 3, 6 and 9 days of storage at 4C. 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 mL of sterile saline (0.85%). Then, the homogenized samples were serially diluted with sterile saline (0.85%), and from each diluent, 0.1 mL was spread on respective bacterial media. Plate count agar and eosine methylene blue agar (Difco Laboratories, Franklin Lakes, NJ) were used for total bacterial flora and coliforms, respectively. The plates were incubated at 37C for 48 h, and the microbial counts were expressed as log cfu/g.

2-Thiobarbituric Acid Reactive Substances (TBARS) Value

Lipid oxidation in the samples was measured at 0, 3 and 6 days as TBARS value according to the method of Jung et al. (2011). Nine milliliters of distilled water and 50 µL of butylated hydroxytoluene (7.2% in ethanol) were added to each meat sample (3 g). The mixture was homogenized (Ika Laboratory Equipment, Seoul, Korea) at 16,000 rpm for 20 s. The homogenate (1 mL) was transferred to a test tube and 2 mL of thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA) was added. The test tubes were heated in a water bath at 90C for 15 min, cooled in cold water and then centrifuged (Union 32R, Hanil Co., Ltd., Daegu, Korea) at 2,260 x g for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (DU 530, Beckman Instruments, Inc., Fullerton, CA). The amount of malondialdehyde was calculated using a standard curve prepared from tetraethoxypropane, and the TBARS value was reported as mg malondialdehyde/kg meat.

Sensory Evaluation

The sensory evaluation of seasoned chicken breast meat samples was carried out as three sensory sessions at the first day (day 0) of sampling. The samples $(2.0 \times 3.0 \times 1.5 \text{ cm})$ were pan-fried for 4 min to achieve a core temperature of approximately 72C as measured by a digital thermometer (YF-160A-type-K, YFE, Hsin-Chu, Taiwan) (Alahakoon *et al.* 2014).

Each pan-fried sample was placed in a white plastic tray with randomly coded 3-digit number and provided for evaluation. Water was provided to cleanse the oral cavity between samples. The pan-fried samples were evaluated for color, odor, flavor, taste, tenderness and overall acceptability by seven semitrained panelists who have experience in sensory evaluation of chicken meat more than 1 year. A 9-point hedonic scale (9 = like extremely, 5 = like moderately 1 = dislike extremely) was used in this study.

Statistical Analysis

The whole experimental procedures were in triplicate. Statistical analysis was performed using a one-way analysis of variance by the procedure of general linear model using SAS program (version 9.3, SAS 2011, SAS Institute, Cary, NC). The differences among the mean values were identified using the Student–Newman–Keul multiple range test at a confidence level of P < 0.05. The mean values and standard errors of the means were reported.

RESULTS AND DISCUSSION

Microbial Analysis

The microbial population of marinated chicken breast with or without CPE treated with EB or HP is shown in Table 1. The total aerobic count of nonirradiated control samples increased significantly from 5.25 log cfu/g (day 0) to 9.70 log cfu/g during 9 days of storage. The EB treatment reduced the initial microbial count; however, a higher reduction was observed in samples treated with 2 kGy having aerobic count 2.09 log cfu/g (day 0) compared with others. The total aerobic count of marinated breast without CPE (control) also showed a significant reduction from 5.25 to 3.21 log cfu/g after HP treatment. The combination of EB with CPE significantly decreased the initial bacterial count to nondetectable level. Meanwhile, HP with CPE significantly decreased the aerobic count to 2.17 log cfu/g, while samples having CPE alone had a total aerobic count of 4.48 log cfu/g (day 0). The addition of CPE intensified the reduction of microbial population, both in EB and in HP during the storage period compared with control samples. The combined use of CPE with EB (2 kGy) or HP is an effective means of reducing bacteria from marinated chicken breast meat and prolonging the product shelf life. Table 1 shows that the total aerobic count of samples having CPE with EB or HP was less than 7 log cfu/g, which is

TABLE 1. MICROBIAL POPULATION (LOG CFU/G) OF THE SEASONED CHICKEN BREAST MEAT ADDED WITH CITRUS PEEL EXTRACT AND TREATED BY ELECTRON BEAM IRRADIATION AND HIGH PRESSURE

		Storage				
Treatment		0	3	6	9	SEM*
Control	0 kGy	5.25 ^{az}	7.29 ^{ay}	8.30 ^{ax}	9.70 ^{aw}	0.052
	1 kGy	3.42 ^{cz}	5.37° ^y	6.06 ^{cx}	6.86 ^{cw}	0.042
	2 kGy	2.09 ^{ez}	3.86 ^{ey}	4.48 ^{ex}	5.47 ^{ew}	0.046
Citrus peel extract	0 kGy	4.48 ^{bz}	6.75 ^{by}	7.12 ^{bx}	8.02 ^{bw}	0.042
	1 kGy	3.21 ^{dz}	4.34 ^{dy}	5.01 ^{dx}	5.95^{dw}	0.050
	2 kGy	ND^{fz}	2.41 ^{fy}	3.11 ^{fx}	4.10^{fw}	0.054
	SEM ⁺	0.038	0.038	0.029	0.030	
Control	0.1 MPa	5.25 ^{az}	7.29 ^{ay}	8.30 ^{ax}	9.70 ^{aw}	0.052
	300 MPa	4.48 ^{bz}	5.25 ^{cy}	6.11 ^{cx}	7.27 ^{cw}	0.025
	400 MPa	3.21 ^{dz}	3.92 ^{ey}	4.52 ^{ex}	5.49 ^{ew}	0.025
Citrus peel extract	0.1 MPa	4.48 ^{bz}	6.75 ^{by}	7.12 ^{bx}	8.02 ^{bw}	0.042
	300 MPa	3.32 ^{cz}	4.82 ^{dy}	5.27 ^{dx}	6.37 ^{dw}	0.036
	400 MPa	2.17 ^{ez}	2.70 ^{fy}	3.26 ^{fx}	4.53 ^{fw}	0.047
	SEM^+	0.034	0.018	0.024	0.023	

* Standard errors of the mean (n = 12).

+ Standard errors of the mean (n = 18).

^{a-f} Values with different letters within the same column differ significantly (P < 0.05).

 $^{\rm w-z}$ Values with different letters within the same row differ significantly (P < 0.05).

ND, not detected.

considered meat as spoiled when the total aerobic count exceed this level (Gyamfi *et al.* 2012; Bae *et al.* 2014) throughout the entire storage period (9 days).

The higher inhibitory effect was observed for 2 kGy EB treatment and 400 MPa HP in samples with and without CPE during the entire period of storage (9 days) compared with other samples. The effectiveness of EB in reducing the total aerobic bacterial count in meat products has been proven by various researchers; Heath et al. (1990) found that an EB dose as low as 1 kGy is effective in reducing the total number of aerobic organisms by 2-3 logs in chicken breasts and thighs. Lewis et al. (2002) observed that no microbial populations were detected after chicken breast meat samples were irradiated with 1.8 kGy. Results of Luchsinger et al. (1996) indicated that an EB dose of 2.5 kGy resulted in a 4-5 log reduction of aerobic plate counts in boneless pork chops. The shelf life of marinated loin slices was extended from 7 to 16 days with the application of 1 and 2 kGy of EB irradiation, respectively (Garcia-Marquez et al. 2012).

However, the rate and the inactivation kinetics of microorganisms under HP depends on the type of microorganism, level of pressure, time of treatment, temperature, pH, water activity and food composition (Hugas *et al.* 2002). It is obvious that complex food matrices such as meat and milk rich in carbohydrates and proteins increase the pressure resistance of some microorganisms (Simpson and Gilmour 1997). The results regarding the effects of EB and HP on the TBARS value of marinated chicken breast meat with or without CPE are presented in Table 2. The addition of CPE was not effective to control lipid oxidation in the marinated breast meat during refrigerated storage. EB at both 1 and 2 kGy and HP at 300 and 400 MPa resulted in higher TBARS values compared with the nontreated samples during day 3 and day 6 (P < 0.05). The samples with CPE at 2 kGy showed the highest TBARS value at 0 day, which further increased up to day 3 and then showed a decline at day 6. The same trend was observed in all samples treated with EB.

It is generally accepted that lipid oxidation is the primary process responsible for quality deterioration during storage as a result of the negative impact on flavor, color, texture and nutritional value (Kim *et al.* 2013). The development of rancidity in meat by lipid oxidation began at the time of slaughter and continues during storage. In addition, free radicals produced during irradiation consequently trigger chemical changes of irradiated meats such as lipid and/or protein oxidation (Kim *et al.* 2013). Furthermore, the reaction of meat components with radiolytic free radicals may result in sulfur volatiles or carbon monoxide in meat, which interfere with sensory qualities (Ahn 2002). Additionally, the breakdown products of lipid oxidation, including

TABLE 2. TBARS VALUES (MG MALONDIALDEHYDE/KG MEAT) OFTHE SEASONED CHICKEN BREAST MEAT ADDED WITH CITRUS PEELEXTRACT AND TREATED BY ELECTRON BEAM IRRADIATION ANDHIGH PRESSURE

		Storage			
Treatment		0	3	6	SEM*
Control	0 kGy	1.22 ^{bx}	1.41 ^{dx}	0.87 ^{cy}	0.079
	1 kGy	1.31 ^{by}	1.93 ^{bx}	1.03 ^{bcy}	0.093
	2 kGy	1.35 ^{by}	2.23 ^{ax}	1.28 ^{ay}	0.039
Citrus peel extract	0 kGy	1.42 ^b	1.30 ^d	1.26ª	0.059
	1 kGy	1.41 ^{bx}	1.59 ^{cx}	1.15 ^{aby}	0.061
	2 kGy	1.69 ^{ax}	1.37 ^{dy}	1.24 ^{ay}	0.047
	SEM ⁺	0.081	0.057	0.055	
Control	0.1 MPa	1.22×	1.41 ^{bx}	0.87 ^{by}	0.079
	300 MPa	1.08 ^y	1.90 ^{ax}	1.16 ^{ay}	0.074
	400 MPa	1.38	1.28 ^b	1.18ª	0.123
Citrus peel extract	0.1 MPa	1.42	1.30 ^b	1.26ª	0.059
	300 MPa	1.29 ^{xy}	1.40 ^{bx}	1.16 ^{ay}	0.047
	400 MPa	1.39	1.35⁵	1.23ª	0.090
	SEM ⁺	0.101	0.080	0.061	

* Standard errors of the mean (n = 9).

+ Standard errors of the mean (n = 18).

 $^{\rm a-d}$ Values with different letters within the same column differ significantly (P < 0.05).

 $^{x-y}$ Values with different letters within the same row differ significantly (P < 0.05).



FIG. 1. SENSORY SCORES OF THE SEASONED CHICKEN BREAST MEAT ADDED WITH CITRUS PEEL EXTRACT AND TREATED BY ELECTRON BEAM IRRADIATION

Note: Values with different letters within the same sensory parameter differ significantly (P < 0.05). C, control; CP, citrus peel extract was added.

aldehydes, ketones, alcohols, hydrocarbons and furans, can cause flavor deterioration in irradiated meat and meat products (Ahn 2002).

Furthermore, irradiation has been reported to increase the TBARS value in different meat species in different packaging and storage conditions (Hampson *et al.* 1996). Brito *et al.* (2002) stated irradiation dose and presence of oxygen as the major causes of lipid oxidation due to irradiation. Lewis *et al.* (2002) stated that TBARS value of chicken breast fillets subjected to 1 and 1.8 kGy were greater than that of control samples over the storage and it further increased as storage time increased.

HP had no significant effect on the TBARS value at day 0, irrespective of CPE addition. However, significant effects of HP on the TBARS value were observed with samples treated at 300 MPa at day 3 and day 6 and 400 MPa at day 6. The samples with CPE showed no significant difference in the TBARS values between samples treated at 300 and 400 MPa during storage period. However, samples without CPE showed a difference in TBARS value between the samples treated at 300 and 400 MPa at day 3 of storage. Moreover, TBARS values of samples treated with HP at 300 MPa showed an increase from day 0 and then decreased at day 6. In several studies, lipid oxidation was not increased immediately after the pressure treatment but induced during sub-

sequent storage (Orlien *et al.* 2000; Beltran *et al.* 2003). However, our findings showed that HP did not significantly increase the TBARS values over the storage period and no significant (P > 0.05) change occurred immediately after the HP in contrast to nontreated samples.

Degeneration of heme-containing proteins causes the lipid oxidation reaction during HP (Jung *et al.* 2013; Kruk *et al.* 2014). Generally, 500 MPa is the critical pressure that initiates lipid oxidation in chicken breast fillet (Orlien *et al.* 2000). However, Cheah and Ledward (1996) stated that the changes leading to catalysis of lipid oxidation in pressure processed meat were initiated at around 300 MPa at room temperature. Similar results were observed in the current study; both 300 and 400 MPa had a significant effect on the TBARS value at day 6 of storage. However, Ma *et al.* (2007) reported that pressure treatments of 600 and 800 MPa were the critical pressure levels to induce increased rates of lipid oxidation in chicken muscle and further clarified that chicken muscle was more stable against HP than did beef.

Sensory Evaluation

Figures 1 and 2 present the sensory scores of the seasoned chicken breast meat with and without CPE and treated by



FIG. 2. SENSORY SCORES OF THE SEASONED CHICKEN BREAST MEAT ADDED WITH CITRUS PEEL EXTRACT AND TREATED BY HIGH PRESSURE Note: Values with different letters within the same sensory parameter differ significantly (P < 0.05). C, control; CP, citrus peel extract was added.

EB or HP. No marked effects were observed (P > 0.05) in the sensory attributes of seasoned chicken breast meat treated with EB (1 and 2 kGy) and HP (300 and 400 MPa) compared with control samples. However, all the sensory qualities tested in both EB- and HP-treated seasoned chicken breast meat were affected by CPE addition, except color and tenderness. The samples with CPE without EB or HP treatment also had lower scores for sensory quality attributes, while EB and HP treatments have lesser impacts on sensory those characteristics as compared to CPE addition. This may be attributed to the unfamiliar taste reported by the semitrained panel of judges during the sensory evaluation. The CPE addition was categorized as unfamiliar, but not in the rejection category from the comments of the sensory panelists (data not shown). Meat samples treated with CPE and EB (2 kGy) had the lowest scores (P < 0.05) for odor, flavor and overall acceptability, whereas those treated with CPE and HP (400 MPa) showed the lowest scores (P < 0.05) for color and odor in HP-treated seasoned chicken meat.

Hayman *et al.* (2004) observed no detrimental effects of HP on sensory qualities of various meat products even at 600 MPa. Crehan *et al.* (2000) stated that HP had no significant influence on the flavor characteristics of food. However, Rivas-Canedo *et al.* (2009) showed that minced beef and chicken breast subjected to HP (400 MPa) resulted

in significant changes in some volatile compounds, alcohols and aldehydes in processed meats, which had an impact on the flavor and aroma. On the contrary, irradiation can induce oxidative deterioration of fatty acids and consequently generate off-flavors and off-odors that cause changes in consumer acceptability (Carrasco *et al.* 2005). Currently, various methods such as modified atmosphere packaging, low temperature irradiation and addition of antioxidants are implemented in order to prevent the generation of off-flavor in irradiated meat and meat products (Brewer 2009).

Combination of CPE with EB and HP is an effective approach to improve the microbial quality of seasoned chicken meat as CPE is an effective antimicrobial agent. However, sensorial quality should be taken into consideration prior to implementation as an alternative in the food industry. Therefore, the adverse effects of CPE on sensorial properties should be minimized by developing the formulation or changing the extraction procedure to avoid mixing of unfamiliar flavor and taste.

CONCLUSIONS

EB and HP combined with CPE are effective means of extending the shelf life of seasoned chicken breast meat.

Even though the synergistic effect of CPE with EB and HP is a very useful approach to enhance the microbiological quality of seasoned chicken breast meat, some adverse effects of CPE addition on flavor and odor attributes were found. Therefore, the development of formulation or extraction procedure of CPE is needed to avoid sensory defects.

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