Effects of bee products (propolis, royal jelly and honeybee comb extracts) on physico-chemical and storage characteristics of pork patties

GYUTAE PARK – HYUNSU CHOI – JINHO CHO – SEYEON CHANG – HWAYONG LEE – JUNGSEOK CHOI

Summary

In this study, effects of bee products (propolis, royal jelly and honeybee comb extracts) added to pork patties on physico-chemical and storage characteristics were investigated. In the study, chemical composition, pH, water holding capacity, cooking loss, texture profile and sensory attributes were analysed regarding the quality of the patties. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity, 2-thiobarbituric acid reactive substances, total microbial counts and volatile basic nitrogen were determined after 0, 3 and 7 days of refrigeration at 4 ± 1 °C. As a result, patties added with bee products tended to show better water holding capacity and cooking loss than those added with ascorbic acid. The addition of bee products improved DPPH radical-scavenging activity and volatile basic nitrogen during 7 days of storage. However, when patties were added with propolis and royal jelly, the flavour deteriorated due to their unique aroma and taste. These results indicate that bee products can be used as additives in patties, but the amount of use should be considered.

Keywords

additive; antioxidant; bee product; meat product; physico-chemical characteristic

Meat products are very susceptible to oxidation and deterioration during storage [1]. Lipid oxidation, which is one of the main causes of meat quality loss, forms several compounds with potentially negative effects on meat quality [2]. Overall, these reactions can lead to undesirable changes in sensory (e.g., colour, texture and flavour) and nutritional properties of meat and meat products [3]. The negative effects are usually delayed by the addition of additives such as antioxidants. However, despite the excellent efficacy and high stability of synthetic antioxidants, concerns about their safety have increased, resulting in increased interest in natural antioxidants [4]. Bee products are valuable sources of biologically active substances [5]. Among bee products, the most famous and widely recognized product is honey [6]. However, in addition to honey, there are several other types of bee products, including pollen, propolis, royal jelly and beeswax. They can serve as functional foods and important sources of physiologically active compounds [7].

Honeybee comb is a non-toxic natural resinous by-product of beekeeping. Honeybee combs contain bee products propolis, royal jelly, honey and pollen. These are known to have various physiological and biochemical properties, as well as functional properties such as antibacterial, antioxidant, antimutagenic, antitumor or anti-inflammatory effects [8]. Propolis is a generic term for resinous substances accumulated by bees from various types of plants [9]. Although the main chemical component of propolis is derived from plant-produced resins, there is evidence that β-glucosidase, a secretion from glands of bees, could potentially be present in the propolis [10]. β-Glucosidase is responsible for enabling propolis to contain large amounts of flavonoids through an enzymatic process that enables rapid hydrolysis of flavonoid glycoside [11]. Phenolic compounds, esters, flavo-

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noids, terpenes, beta steroids, aromatic aldehydes and alcohols are important organic compounds present in propolis [12]. Phenolic acids and flavonoids are main components responsible for the antioxidant activity of propolis [13]. Royal jelly is a yellowish-white, creamy, acidic secretion from mandibular and hypopharyngeal glands of young worker bees [14]. Royal jelly contains the royal jelly major protein 1-9 family. Additionally, royal jelly also contains antimicrobial peptides [15] and free amino acids such as histidine or serine [16]. Currently, the development of nutraceuticals and functional foods is increasing, with many studies investigating direct health benefits and pharmacological properties of bee products. However, there are few cases in which bee products are mixed and used. Therefore, an experiment was conducted to determine their value of use as a food compared to ascorbic acid, a representative antioxidant. The purpose of this study was to determine effects of adding beekeeping products (honeybee comb, propolis and royal jelly) on physico-chemical properties and storage stability of pork patties.

MATERIALS AND METHODS

Preparation of honeybee comb extracts

Honeybee comb after harvesting honey was extracted with 95% (v/v) ethanol (food-grade) for one week. Ethanol was then evaporated using a rotary vacuum concentrator NE-1 (Eyera, New York, New York, USA). Concentrates of honeybee comb extracts (HCE) remaining after evaporation were lyophilized and stored for a maximum of 2 months.

Phenolic acids analysis

Phenolic acids in the extracts of bee products were analysed using reverse-phase high performance liquid chromatography (HPLC) based on the method of Dimitrova et al. [17] with some modifications as described below. Chromatographic analyses were carried out using a Young Lin HPLC equipment, series YL-9100 (Younglin, Anyang, South Korea), equipped with a quaternary pump, an autosampler (YL9150), a degasser and a YL9160 3 spectrophotometric detector set at 220 nm and 280 nm. Spherisorb ODS2 column of 5 μm, 4.6 mm × 250 mm was used

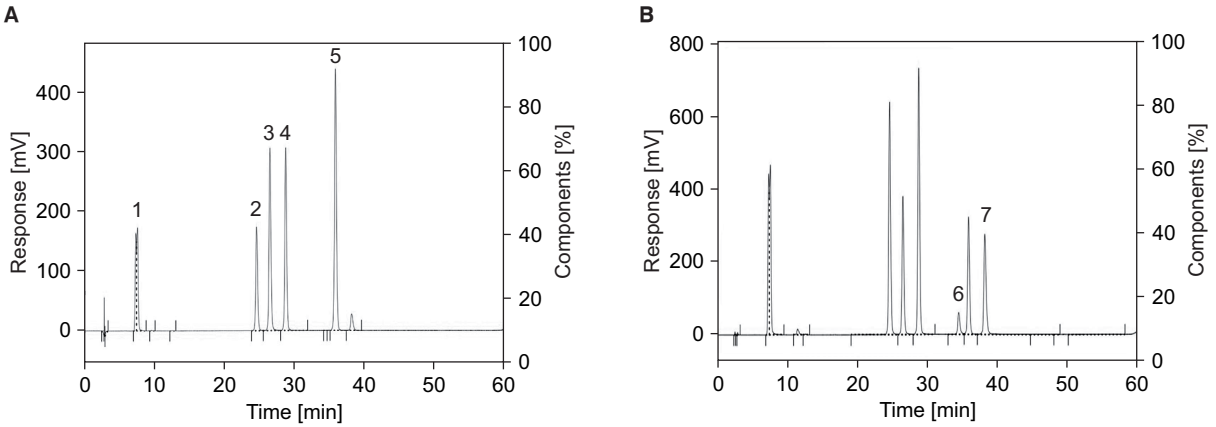

Fig. 1. Chromatograms of a standard solution of phenolic acids.

A – detection at 280 nm, B – detection at 220 nm.
Key to peak identities: 1 – gallic acid, 2 – vanillic acid, 3 – caffeic acid, 4 – syringic acid, 5 – *p*-coumaric acid, 6 – phenylacetic acid, 7 – benzoic acid.

Tab. 1. Calibration parameters of phenolic compound standards.

Peak No.	Compounds	Regression equation	R^2	Retention time [min]
1	Gallic acid	$y = 26.161x - 9.5186$	0.9997	7.45
2	Vanillic acid	$y = 34.018x - 196.89$	0.9888	24.677
3	Caffeic acid	$y = 59.898x - 198.55$	0.9962	26.570
4	Syringic acid	$y = 57.766x - 192.86$	0.9955	28.837
5	*p*-Coumaric acid	$y = 86.705x - 26.48$	0.9998	35.983
6	Phenylacetic acid	$y = 14.349x - 66.366$	0.9981	34.672
7	Benzoic acid	$y = 63.073x + 119.32$	0.9936	38.420

(Waters, Milford, Massachusetts, USA). The flow rate was fixed at 1.0 ml·min^{-1}. The mobile phase was composed of 99.5% (v/v) methanol (Samchun, Pyeongtaek, South Korea) and 20 mmol·l^{-1} potassium dihydrogen phosphate buffer (adjusted to pH 2.92 with ortho-phosphoric acid), which was filtered using 0.22 μm filter (Corning, New York, New York, USA). Elution conditions involved a gradient of binary mobile phase: solvent A (20 mmol·l^{-1} potassium dihydrogen phosphate buffer at pH 2.92) and solvent B (methanol). The elution program commenced with 3 % B followed by 45 % B at 55 min and 100 % B at 65 min. The following authentic standards of phenolic acidd were used: gallic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, phenylacetic acid and benzoic acid. Determination by HPLC was performed in triplicate (Fig. 1, Tab. 1).

Preparation of treatments

Ground pork hind legs were purchased from a local commercial butcher and blood was removed. Salt, propolis (Yangbongnh, Seoul, South Korea), royal jelly (Ohfarm, Seoul, South Korea), HCE, and ascorbic acid were mixed with distilled water so that the temperature of the mixture was maintained at 10 °C for 5 min using a mixer. Patties (100 g each) to be used for all treatments were prepared in a circular shape. The composition ratio of each treatment is shown in Tab. 2. In the experimental design, control (CON) was the basic patty condition. Other conditions were designed to include representative antioxidants ascorbic acid and bee products in the CON patty. Positive control (P-CON) was added ascorbic acid to CON, treatment 1 (T1) was added ascorbic acid and bee products to CON and treatment 2 (T2) was added bee products to CON. The concentration of the added bee products was designed to be a concentration that was effective in previous preliminary experiments. Prepared patties were wrapped without heating and stored in a refrigerator at 4 °C. Three refrigerated pork patties from each treatment group were analysed according to the storage period (0, 3, and 7 days). The experiment was repeated three times.

Chemical composition determination

Moisture, protein, fat and ash content of 0-day patties were determined according to AOAC 934.01 (moisture), 992.15 (protein), 960.39 (fat) and 920.153 (ash) [18]. To measure moisture, 1 g of sample was placed on an aluminium weighing dish and heated in a dry oven (Samheung, Seoul, South Korea) at 105 °C for 16 h. It was then calculated using the weight after heating and the weight before heating. For the protein, 0.5 g of sample and 25 ml of sulfuric acid were added to the flask, and the ammonia component was adsorbed using boric acid. After that, titration was performed using sulfuric acid. For crude fat estimation, a 0.5 g sample was homogenized in 25 ml of Folch solution (chloroform-methanol, 2 : 1) and left in a refrigerator at 4 °C for 24 h. The sample was filtered through Whatman No. 2 paper (Whatman, Maidstone, United Kingdom) and cleaned with 5 ml of Folch solution. After mixing, 10 ml of distilled water was added to the filtrate and the sample was centrifuged at 125 ×*g* at 4 °C for 20 min. After removing the separated upper layer consisting of water and ethanol using a pipette, chloroform was evaporated overnight in a hood and the weight was measured. For ash content measurement, 0.3 g of sample was placed in a crucible and placed in a muffle furnace (Jeiotech, Daejeon, South Korea) at 540 °C for 10 h. Then, it was cooled for 1 h and calculated using the weight before and after incineration.

Tab. 2. Formulation of pork patties added with bee products.

Component	CON	P-CON	T1	T2
Pork [g]	100	100	100	100
Distilled water [g]	10	10	10	10
Salt [g]	1.5	1.5	1.5	1.5
Ascorbic acid [g]	–	1.015	1.015	–
Propolis [g]	–	–	0.3045	0.3045
Royal jelly [g]	–	–	1.015	1.015
Honeybee comb extract [g]	–	–	1.015	1.015

CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

pH

A volume of 90 ml of distilled water was added to 10 g of each pork patty sample to measure the pH value. All samples were homogenized for 30 s using a homogenizer (Bihon Seiki, Ace, Japan), followed by measurement of pH using a pH meter Delta 340 (Mettler Toledo, Columbus, Ohio, USA).

Water holding capacity and cooking loss

To measure cooking loss (*CL*), the patty was heated using a water bath (Hanyang Science Lab, Seoul, South Korea) until its internal temperature reached 70 °C. After cooling for 10 min, the weight was measured and *CL* was expressed as a portion of the initial weight. Water holding ca-

pacity (*WHC*) was measured by a modified centrifugation method of Park et al. [19]. After measuring 0.5 g of each sample into a tube, it was heated at a constant temperature of 80 °C in a water bath for 20 min. After allowing to cool for 10 min, the sample was centrifuged for 10 min (10 °C) at 83 ×*g*. The weight was measured and calculated as follows:

$$WHC = \left(\frac{a-b}{a}\right) \times 100 \quad (1)$$

where *a* is total moisture, *b* is free moisture (FM).

$$FM = \left(\frac{x-y}{z \cdot v}\right) \times 100 \quad (2)$$

where *x* is weight before centrifugation, *y* is weight after centrifugation, *z* is weight of sample and *v* is fat coefficient (*FC*).

$$FC = 1 - \left(\frac{F}{100}\right) \quad (3)$$

where *F* is fat (expressed in percent).

Meat colour measurement

Surface meat colour of each pork patty was measured with a spectrophotometer model JX-777 (Color Techno System, Tokyo, Japan). A white fluorescent lamp D65 (Color Techno System) was used to determine L^*, a^*, and b^* values with a Hunter Lab colour system (L^* is brightness; a^* is redness; b^* is yellowness).

Sensory test

A sensory test was performed by seven evaluation panelists in the Department of Animal Science, Chungbuk National University (Cheongju, South Korea). All patties were cooked using a pre-heated pan until the internal temperature reached 72 ± 1 °C. They were heated at this temperature for 7 min. Patties were cut into blocks with a thickness of 1.5 cm × 1.5 cm × 1.5 cm. Each patty was placed on a white plate. The sensory test was conducted at room temperature (18–21 °C). After eating one sample, evaluation panelists were asked to rinse their mouths with water and eat the next sample after waiting for 1–2 min. Evaluation factors consisted of colour, flavour, off-odour, tenderness, juiciness and overall preference. The evaluation was performed using a 5-point rating method. Each item was scored from 1 point (light colour, worst flavour, more off-odour, less tenderness, less juiciness, worst overall acceptance) to 5 points (dark colour, most flavour, less off-odour, more tenderness, more juiciness, best overall acceptance).

2-Thiobarbituric acid reactive substances

2-Thiobarbituric acid reactive substances (*TBARS*) value was measured using a modified extraction method of Park et al. [19]. Briefly, each sample (10 g) was homogenized with 15 ml of 100 g·l^{-1} perchloric acid and 25 ml of distilled water using a homogenizer Ultra-Turrax T25 (Ika, Staufen, Germany) on ice. After homogenization, the whole eluate was transferred to Whatman No. 2 filter paper. Using a pipette, 5 ml of each filtrate and 0.02 mol·l^{-1} 2-thiobarbituric acid (TBA) solution were transferred into a numbered tube and the lid was closed. After mixing well using a vortex mixer, 5 ml each of distilled water and 0.02 mol·l^{-1} TBA solution were mixed and used as a blank. After mixing, the surface was sealed with parafilm and the tube was placed in a tube rack. After incubation in a refrigerator at 4 °C in the dark for 16 h, absorbance was measured at 529 nm using a microplate spectrophotometer Mobi (Microdigital, Seongnam, South Korea).

Total microbial counts

Total microbial counts were determined using plating. A 1 g·l^{-1} peptone solution (90 ml) was added to 10 g of the sample and homogenized for 30 s with a stomacher. Then, the serially diluted samples were inoculated onto plate count agar (PCA) medium (Becton Dickinson, Franklin Lakes, New Jersey, USA) and incubated at 37 °C for 48 h. After the incubation was completed, the colonies were counted using a colony counter. The total number of microorganisms was expressed as logarithm of colony forming units per gram.

Antoxidant activity

Antioxidant activity (*AA*) of each sample was determined using a modified DPPH free radical-scavenging assay. A total of 5 g of each patty and 45 ml of 99.5% (v/v) methanol (Samchun Pure Chemical, Seoul, South Korea) were homogenized for 1 min with a homogenizer Ultra-Turrax T25. The solution was filtered through a Whatman No. 2 filter paper to remove impurities. Then, samples, blanks and references were prepared as follows: sample, 2 ml of solution, 1 ml of 0.02 mmol·l^{-1} DPPH solution (Biozoa Biological Supply, Seoul, South Korea) and 2 ml of methanol; blank, 5 ml of methanol; and reference: 1 ml of DPPH solution and 4 ml of methanol. Samples were left at room temperature (18–21 °C) in a dark room for 20 min. The absorbance of each solution was measured at 517 nm using a microplate spectrophotometer Mobi. *AA* of the patty sample against DPPH radical was calculated as follows:

$$AA = \left[1 - \left(\frac{c}{d}\right) - e\right] \times 100 \quad (4)$$

where c is absorbance of sample, d is absorbance of reference sample and e is absorbance of blank sample.

Volatile basic nitrogen

The method of Kim and Kim [20] was used with some modifications to measure volatile basic nitrogen (*VBN*) content. Distilled water (90 ml) was added to 10 g of the sample and homogenized using a homogenizer Ultra-Turrax T25. The homogenate was filtered through Whatman No. 2 filter paper. The filtrate (1 ml) was placed in the outer chamber of the conway unit and 1 ml of 0.01 mol·l^{-1} boric acid solution and 3 drops of the indicator (methyl red with bromocresol green) were added to the inner chamber. After applying white vaseline to the adhesive part of the lid and closing the lid, 1 ml of 3.6 mol·l^{-1} K_2CO_3 was injected into the outer chamber, which was then immediately sealed. The vessel was stirred horizontally and incubated at 37 °C for 2 h. After incubation, boric acid solution in the inner chamber was titrated with 0.01 mol·l^{-1} H_2SO_4. The *VBN* level was expressed as milligrams per kilogram sample.

$$VBN = \frac{(f - g) \cdot 0.98 \cdot 28.014 \cdot 100}{h} \quad (5)$$

where f is amount of H_2SO_4 injected, g is amount of H_2SO_4 injected into the blank and h is amount of sample.

Statistical analysis

All experiments were repeated at least three times. All statistical analyses were performed through the General Linear Model procedure of the SAS program (Sas Institute, Cary, North Carolina, USA). The significance (at $p < 0.05$) was determined using Duncan's multiple test for comparing means of treatment groups.

RESULTS AND DISCUSSION

Phenolic compounds of bee products

Fig. 2 and Tab. 3 show the results on phenolic compounds in bee products. Six phenolic compounds were isolated from propolis, namely, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, phenylaceate (excluding gallic acid) and benzoic acid. Four phenolic compounds were isolated from royal jelly: gallic acid, *p*-coumaric acid, phenylaceate and benzoic acid. Vanillic acid, caffeic acid and syringic acid were not detected. Phenolic compounds of HCE included vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, phenylaceate (excluding gallic acid) and benzoic acid. Commonly found phenolic compounds in three honeycomb by-products were *p*-coumaric acid, phenylaceate and benzoic acid. The antioxidant activity of a bee product depends on a wide range of components including phenolics, peptides, organic acids, enzymes, Maillard reaction products and other trace compounds. However, among them, phenolic compounds are known to have very important contribution to antioxidant activity [21]. The antioxidant activity of a bee product is mainly provided by phenolic compounds. All three bee products examined were confirmed to contain four or more phenolic compounds. Although most of the phenolic compounds investigated were detected in small amounts, it was found that a mixture of bee products might have the potential to be used as a natural antioxidant product in food sector.

Chemical composition

Tab. 4 shows data on chemical composition of pork patties added with bee products. There was no significant difference in moisture, protein or ash content between the control group and the treatment group. Regarding fat proportion, patties treated with bee products showed significant differences from the control group. According to chemical composition specifications of propolis, crude fat was 86.4 %, soluble nitrogen-free material was 7.3 %, crude protein was 2.7 %, ash was 1.1 % and crude fibre was 0.2 % [22]. In addition, the chemical components of royal jelly were measured at the level of protein, fat, sugar and ash at 12.8 % to 15.1 %, 7.9 % to 9.1 %, 11.0 % to 12.3 %, and 0.9 % to 1.5 %, respectively [23]. In the case of the chemical composition specifications of HCE, crude protein, crude fibre, ether extract, ash and nitrogen free extract were 9.4 %, 0.5 %, 54.9 %, 1.8 %, and 23 %, respectively [24]. So, it is considered that the difference in fat content of the patties was caused by the addition of the bee product.

Water holding capacity, cooking loss and pH

Tab. 5 shows data on *WHC*, *CL* and pH of pork patties supplemented with bee products. P-CON and T1 showed significantly low pH values. It was considered that the pH decreased as ascorbic acid was added to P-CON and T1. *WHC* also showed lower values in P-CON and T1. Puolanne and Peltonen [25] reported that when pH was decreased from 5.7 to 4.5 in meat sausage, *WHC* de-

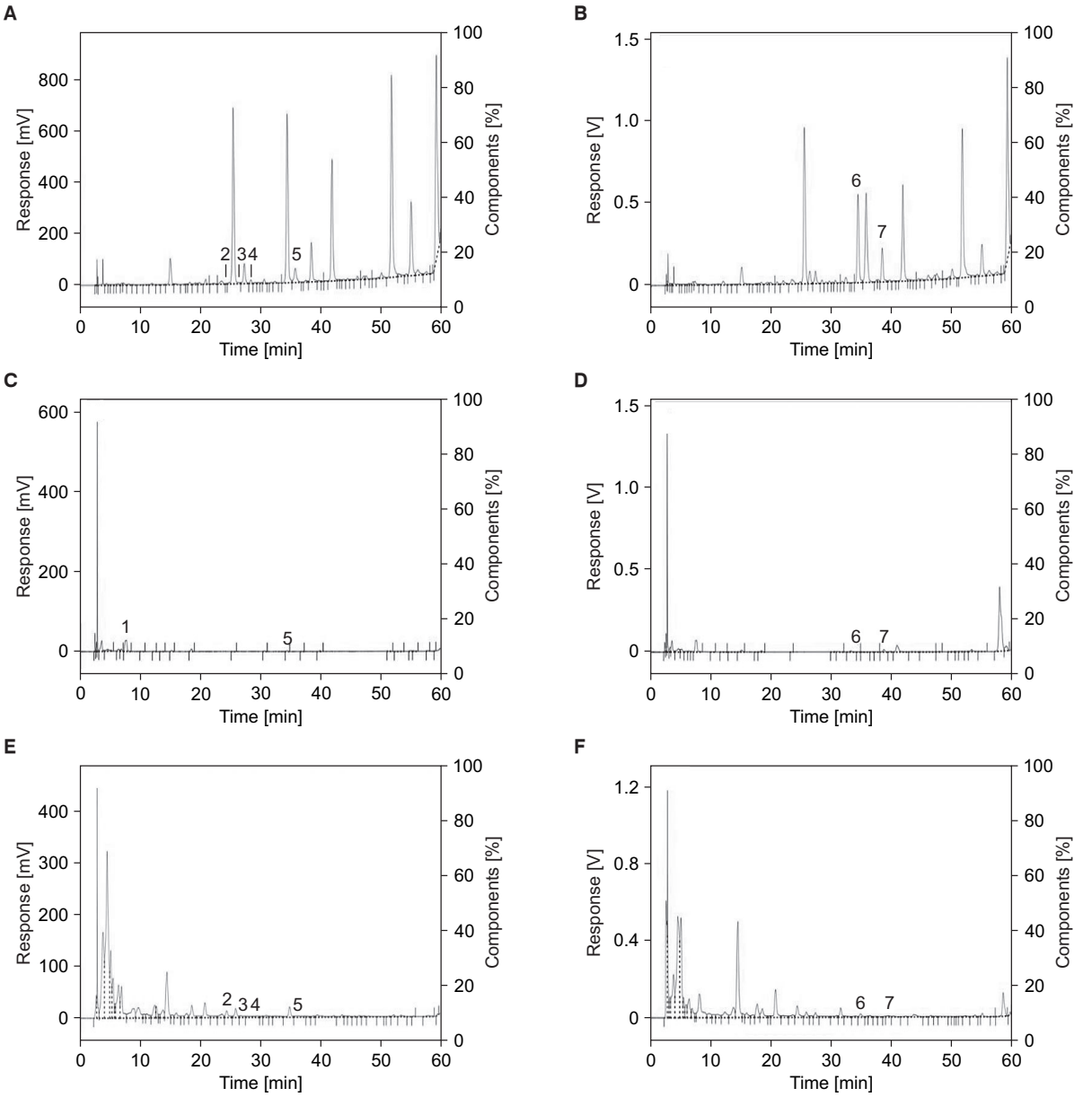

Fig. 2. Phenolic acids in bee products separated using high performance liquid chromatography.

A – propolis analysis with detection at 280 nm, B – propolis analysis with detection at 220 nm, C – royal jelly analysis with detection at 280 nm, D – royal jelly analysis with detection at 220 nm, E – honeybee comb extract analysis with detection at 280 nm, F – honeybee comb extract analysis with detection at 220 nm.
Key to peak identities: 1 – gallic acid, 2 – vanillic acid, 3 – caffeic acid, 4 – syringic acid, 5 – *p*-coumaric acid, 6 – phenylacetic acid, 7 – benzoic acid.

Tab. 3. Concentration of phenolic compounds in bee products.

Phenolic compounds	Propolis	Royal jelly	Honeybee comb extracts
Gallic acid [$\mu g \cdot l^{-1}$]	ND	27.803 ± 0.276	ND
Vanillic acid [$\mu g \cdot l^{-1}$]	7.571 ± 1.934	ND	17.428 ± 0.466
Caffeic acid [$\mu g \cdot l^{-1}$]	3.796 ± 0.833	ND	7.770 ± 1.667
Syringic acid [$\mu g \cdot l^{-1}$]	5.140 ± 0.176	ND	6.726 ± 1.707
p-Coumaric acid [$\mu g \cdot l^{-1}$]	24.976 ± 1.124	6.162 ± 0.097	7.863 ± 0.703
Phenylacetic acid [$\mu g \cdot l^{-1}$]	729.737 ± 47.791	6.273 ± 0.517	40.387 ± 2.906
Benzoic acid [$\mu g \cdot l^{-1}$]	61.774 ± 3.784	3.030 ± 0.069	3.759 ± 0.392

ND – not detected.

Tab. 4. Chemical composition of pork patties added with bee products.

	Treatments			
	CON	P-CON	T1	T2
Moisture [%]	65.7 ± 1.3	66.8 ± 1.4	64.5 ± 3.5	63.5 ± 3.1
Crude ash [%]	1.4 ± 0.2	1.2 ± 0.1	1.2 ± 0.3	1.1 ± 0.1
Crude fat [%]	14.2 ± 1.0 ^{ab}	12.7 ± 0.8 ^{b}	15.0 ± 1.2 ^{a}	15.8 ± 1.5 ^{a}
Crude protein [%]	18.6 ± 0.5	19.3 ± 0.7	19.2 ± 2.2	19.5 ± 2.1

Means with different superscripts within the same row differ significantly ($p < 0.05$, $n = 3$).
CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

Tab. 5. Water holding capacity, cooking loss and pH of pork patties added with bee products.

	Treatments			
	CON	P-CON	T1	T2
Water holding capacity [%]	62.7 ± 0.7 ^{a}	52.4 ± 1.9 ^{b}	49.7 ± 3.2 ^{b}	61.2 ± 2.5 ^{a}
Cooking loss [%]	24.0 ± 1.4 ^{b}	36.7 ± 1.2 ^{a}	37.5 ± 1.3 ^{a}	25.2 ± 4.3 ^{b}
pH	5.47 ± 0.11 ^{a}	4.63 ± 0.03 ^{b}	4.61 ± 0.03 ^{b}	5.46 ± 0.02 ^{a}

Means with different superscripts within the same row differ significantly ($p < 0.05$, $n = 3$).
CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

Tab. 6. Meat colour of pork patties added with bee products.

	Treatments			
	CON	P-CON	T1	T2
L^* (brightness)	73.16 ± 3.24 ^{b}	80.94 ± 2.00 ^{a}	83.19 ± 5.07 ^{a}	73.88 ± 1.31 ^{b}
a^* (redness)	15.07 ± 0.13 ^{a}	13.24 ± 0.63 ^{b}	10.30 ± 1.11 ^{c}	13.26 ± 0.43 ^{b}
b^* (yellowness)	15.58 ± 2.13	16.01 ± 1.53	14.91 ± 1.53	16.49 ± 0.52

Means with different superscripts within the same row differ significantly ($p < 0.05$, $n = 3$).
CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

creased regardless of salinity. On the other hand, *WHC* of the treatment group without addition of ascorbic acid showed a significant increase. *CL* showed high values in P-CON and T1. This appeared to be due to the low *WHC*. According to a study by HUGHES et al. [26], water released can be described as drip, purge, weep, exudate or cook loss.

Meat colour

Tab. 6 shows meat colour results of pork patties supplemented with bee products. P-CON and T1 showed higher brightness values than other treatment groups. A study by GIROUX et al. [27] showed that the addition of 5 g·kg^{-1} ascorbic acid significantly increased brightness values of beef patties. Therefore, the difference in lightness value of pork patties was considered to be due to the effect of ascorbic acid. In addition, redness value was low in all treatment groups except for the control group. Among them, T2 with the addition of bee products and ascorbic acid showed the lowest redness. In the case of ascorbic acid, adding 5 g·kg^{-1} to beef patties tended to reduce redness [27]. It was determined that the colour of pork patty was influenced by the colour of ascorbic acid and yellow colour of the bee products. There was no significant difference in yellowness between treatment groups.

Sensory evaluation

Sensory evaluation results of pork patties added with bee products are shown in Tab. 7. In the case of T1 and T2 with the addition of bee products, they showed lower scores of off-odour and flavour items compared to other treatments. As a result, the overall acceptability was affected by the addition of bee products. Propolis usually has a strong, unpleasant taste and odour that impair food palatability [28]. Studies showed that adding 10 g·kg^{-1} or more of propolis to honey can increase its sweetness and bitterness [5]. Royal jelly contains sour and sweet tastes. It is a food with viscosity. In addition, royal jelly has a unique sour smell and spicy taste [29]. Because bee products have strong and unpleasant taste

Tab. 7. Sensory evaluation results of pork patties added with bee products.

	Treatments			
	CON	P-CON	T1	T2
Colour	2.66 ± 0.51	2.83 ± 0.75	2.66 ± 0.81	3.00 ± 1.09
Flavour	3.66 ± 0.81 [a]	3.00 ± 1.41 [a]	1.33 ± 0.81 [b]	1.58 ± 0.80 [b]
Off-odour	3.16 ± 0.40 [a]	3.00 ± 1.09 [a]	1.83 ± 0.98 [b]	2.41 ± 0.91 [ab]
Juiciness	3.00 ± 0.63 [a]	2.50 ± 1.04 [a]	2.33 ± 0.81 [a]	2.75 ± 0.98 [a]
Total preference	3.91 ± 0.66 [a]	2.83 ± 1.12 [b]	1.25 ± 0.41 [c]	2.08 ± 0.86 [bc]

Means with different superscripts within the same row differ significantly ($p < 0.05$, $n = 3$).
CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

Tab. 8. Storage stability of pork patties added bee products.

	Storage [d]	Treatments			
		CON	P-CON	T1	T2
TBARS [mg·kg^{-1}]	0	0.05 ± 0.01 [a]	0.06 ± 0.01 [a]	0.06 ± 0.01 [a]	0.03 ± 0.01 [b]
	3	0.07 ± 0.01 [b]	0.07 ± 0.01 [b]	0.12 ± 0.01 [a]	0.09 ± 0.01 [b]
	7	0.06 ± 0.01 [c]	0.08 ± 0.01 [b]	0.11 ± 0.01 [a]	0.07 ± 0.01 [b]
AA [%]	0	70.9 ± 0.3 [ab]	70.1 ± 0.9 [b]	69.5 ± 0.9 [b]	72.0 ± 0.7 [a]
	3	43.6 ± 3.4 [b]	63.0 ± 0.5 [a]	65.0 ± 0.6 [a]	67.1 ± 2.7 [a]
	7	17.2 ± 2.2 [b]	60.1 ± 1.6 [a]	59.2 ± 1.3 [a]	59.4 ± 1.4 [a]
TMC [log CFU·g^{-1}]	0	7.01 ± 0.04 [a]	5.91 ± 0.02 [b]	6.09 ± 0.03 [b]	6.76 ± 0.02 [a]
	3	7.75 ± 0.03 [a]	6.51 ± 0.03 [b]	6.54 ± 0.05 [b]	7.29 ± 0.04 [a]
	7	7.84 ± 0.02 [a]	6.50 ± 0.03 [b]	6.61 ± 0.04 [b]	7.35 ± 0.04 [a]
VBN [mg·kg^{-1}]	0	70.6 ± 4.1 [b]	68.8 ± 4.1 [b]	78.8 ± 1.6 [a]	82.5 ± 4.2 [a]
	3	87.9 ± 1.5 [a]	76.0 ± 2.7 [c]	86.1 ± 5.7 [ab]	80.6 ± 3.2 [bc]
	7	104.7 ± 1.5 [a]	85.6 ± 0.2 [d]	93.8 ± 1.5 [c]	98.3 ± 3.2 [b]

Means with different superscripts within the same row differ significantly ($p < 0.05$, $n = 3$).
TBARS – thiobarbituric acid reactive substances (expressed as milligrams of malondialdehyde), *AA* – antioxidant activity, *TMC* – total microbial counts, *VBN* – volatile basic nitrogen, CON – control, P-CON – positive control, T1 – treatment 1, T2 – treatment 2.

with unique aroma, it was considered that the low scores of flavour and off-odour were caused by bee products when added to pork patties.

Storage characteristics

As shown in Tab. 8, *TBARS, AA, TMC* and *VBN* were evaluated to determine the storage stability of pork patties added with bee products. In the case of *TBARS*, T2 was significantly lower on Day 0 and T1 was significantly higher on Day 3 and Day 7. Greene and Cumuze [30] reported that when malondialdehyde (MDA) level was 0.6–2 mg·kg^{-1}, an oxidized flavour could be felt upon ingestion. As a result, it is considered that all samples do not have the oxidized flavour when ingested even after a storage period of 7 days. *AA* was significantly higher in T2 at Day 0, but significantly lower in CON at both Day 3 and Day 7. The reason for the high *AA* in P-CON, T1 and T2 was because ascorbic acid added to P-CON and T1 treatment was a representative antioxidant [31]. It was also due to phenolic compounds present in bee products added to T1 and T2 [32]. In the case of *VBN*, Day 0, Day 3 and Day 7 samples all showed significantly lower values in P-CON but significantly higher values in CON. *VBN* is affected by microbial contamination, increasing with an increase in microbial counts [33].

CONCLUSIONS

In this study, physico-chemical properties and storage properties of pork patties added with bee products were investigated. Our study results showed that the addition of bee products did not affect the chemical composition of pork patties. Also, when added bee products, they tended to show better *WHC* and *CL* than when added ascorbic acid. The addition of bee products during 7 days of storage improved DPPH radical-scavenging activity and *VBN*. However, when

propolis and royal jelly were added, the flavour deteriorated due to their unique aroma and taste. As a result of this study, it is thought that the mixed use of bee products would improve *CL*, *WHC* and storage stability of patties compared to ascorbic acid. However, effects on organoleptic properties should be considered.

Acknowledgements

This research was supported by "Regional Innovation Strategy (RIS)" through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021RIS-001).

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Received 21 July 2023; 1st revised 6 September 2023; accepted 29 September 2023; published online 24 January 2024.