

AROMA ACTIVE COMPOUNDS DIFFERENCES OF ROASTED PORK
FROM CP-KUROBUTA PIG AND THREE CROSSBRED PIG
(LARGE-WHITE X LANDRACE X DUROC)

ความแตกต่างของสารประกอบที่ให้กลิ่นในเนื้อสุกรอบจากสุกร ซีพี คูโรบุดะ
และสุกรลูกผสมสามสาย (ลาจไวท์ X แลนด์เรซ X ดูโรค)

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Abstract

Kurobuta pork is well known worldwide for its premium quality and is widely preferred by consumers. However, there is a lack of study on its aroma flavors. In this study, we compared aroma volatile compounds profiles of roasted pork from CP-Kurobuta pigs (CPK) with those of pork from 3-way Landrace x Yorkshire x Duroc crossbred pigs (LYD). Using solid phase microextraction (SPME) and simultaneous distillation and solvent extraction (SDE) combined with GC-MS, 47 volatile compounds in the pork of CPK pigs were identified the dominant amounts of aldehydes, amines and alkenes, while only 20 volatile compounds were detected in LYD pork with dominant amounts of alkenes, alkanes and amines. Using aroma extract dilution analysis (AEDA)-GC-O method, 36 aroma active compounds were determined in CPK pork, while only 9 aroma active compounds were detected in LYD pork. Six out of 36 aroma active compounds of CPK pork were potent aroma compounds including 2-Aziridin ethylamine (sweet roasted pork, honey) (Log₃ FD factor = 2), benzyl aldehyde (roasted pork, almond, smoke), cinamal aldehyde (cinnamon), nonanal (sweet roasted pork, honey, citrus), 1,2-Propanediamine, hydrocyl benzylamine (roasted pork) (Log₃ FD factor = 1). In LYD pork, 2-Aziridin ethylamine (Log₃ FD factor = 1) was the only one potent aroma compound. The results of sensory analysis corresponded with the results of volatile compounds analysis. Panelists detected 6 flavors (roasted pork, smoked pork, heated oil, fresh pork, caramel and honey) in CPK pork, while only 3 flavors (roasted pork, heated oil, fresh pork) were detected in LYD pork. In conclusion, our results show that CPK pork is higher in both number and concentration of the aroma active volatiles than LYD pork. The unique aroma flavor of roasted

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CPK pork and LYD pork are revealed for the first time. These results could be one of the reasons why consumers prefer the Kurobuta pork.

Keywords: Kurobuta pork, Aroma, Volatile

บทคัดย่อ

เนื้อสุกรคุโรบูตะเป็นเนื้อสุกรที่มีชื่อเสียง และเป็นที่ยุ้จักอย่างกว้างขวางทั่วโลกในแง่ของเนื้อสัตว์คุณภาพสูง และได้รับความชื่นชอบอย่างแพร่หลายจากผู้บริโภค แต่การศึกษาในเรื่องกลิ่นรสของเนื้อสุกรชนิดนี้ยังมีอยู่น้อย ดังนั้นในการศึกษาค้นคว้าครั้งนี้ ผู้วิจัยจึงได้ศึกษาเปรียบเทียบรูปแบบของสารประกอบที่ให้กลิ่นในเนื้อสุกรอบจากสุกรซีพีคุโรบูตะ (CPK) และสุกรลูกผสมสามสาย (ลาจไวท์ x แลนด์เรซ x ดุร็อค) (LYD) ด้วยเทคนิค SPME (solid phase microextraction และ SDE (simultaneous distillation and solvent extraction) ร่วมกับ GC-MS (Gas Chromatography - Mass Spectrometry) ผลปรากฏว่า ในเนื้อสุกร CPK สามารถตรวจพบสารประกอบที่ให้กลิ่น 47 ตัว ประกอบด้วยสารหลักๆ ในกลุ่มอัลดีไฮด์ เอมีน และอัลคีน ขณะที่เนื้อสุกร LYD สามารถตรวจพบสารประกอบที่ให้กลิ่น 20 ตัว ประกอบด้วยสารหลักๆ คือ อัลคีน อัลเคน และเอมีน นอกจากนี้ผู้วิจัยยังใช้เทคนิค AEDA (aroma extract dilution analysis) ร่วมกับ GC-O (Gas Chromatography - Olfactory) ในการวิเคราะห์สารประกอบที่ให้กลิ่นดังกล่าวว่า สารใดเป็นสารให้กลิ่นหลัก พบว่า ในเนื้อสุกร CPK มีสารประกอบที่ให้กลิ่นหลัก 36 ตัว ส่วนในเนื้อสุกร LYD มีสารประกอบที่ให้กลิ่นหลัก 9 ตัว ซึ่งในสารประกอบที่ให้กลิ่นหลัก 36 ตัว ของเนื้อสุกร CPK นั้นเป็นสารที่มีความเข้มข้นของกลิ่นสูงอยู่ 6 ตัว ประกอบด้วย 2-Aziridin ethylamine (กลิ่นหวานเนื้อสุกรอบ กลิ่นน้ำผึ้ง) (ค่า Log3 FD factor = 2), benzyl aldehyde (กลิ่นเนื้อสุกรอบ กลิ่นแอลมอนด์ กลิ่นอบควัน), cinamaldehyde (กลิ่นซินนามอนด์), nonanal (กลิ่นหวานเนื้อสุกรอบ กลิ่นน้ำผึ้ง กลิ่นส้ม), 1,2-Propanediamine, hydrocyl benzylamine (กลิ่นเนื้อสุกรอบ) (ค่า Log3 FD factor = 1) ส่วนสารให้กลิ่นที่มีความเข้มข้นสูงในเนื้อสุกร LYD พบเพียงตัวเดียวคือ 2-Aziridin ethylamine (ค่า Log3 FD factor = 1) นอกจากนี้การวิเคราะห์ทางประสาทสัมผัสของเนื้อสุกรทั้งสองกลุ่มในงานวิจัยครั้งนี้ให้ผลสอดคล้องไปในทางเดียวกับผลการวิเคราะห์สารประกอบที่ให้กลิ่น โดยพบว่า ผู้ตรวจชิมเนื้อสุกรสามารถตรวจพบกลิ่นรส 6 ชนิด ในเนื้อสุกร CPK (กลิ่นเนื้อสุกรอบ กลิ่นเนื้อสุกรรมควัน กลิ่นน้ำมันที่โดนความร้อน กลิ่นสุกรสด กลิ่นคาราเมล และกลิ่นน้ำผึ้ง) แต่ตรวจพบกลิ่นรสในเนื้อสุกร LYD เพียง 3 ชนิด (กลิ่นเนื้อสุกรอบ กลิ่นน้ำมันที่โดนความร้อน และกลิ่นสุกรสด) สรุปได้ว่า เนื้อสุกร CPK มีทั้งปริมาณและความเข้มข้นของสารประกอบที่ให้กลิ่นสูงกว่าเนื้อสุกร LYD ซึ่งงานวิจัยในครั้งนี้ได้รายงานกลิ่นหอมที่เป็นเอกลักษณ์ของเนื้อสุกร CPK และ LYD เป็นครั้งแรกจึงอาจนำไปสู่การอธิบายเหตุผลที่ว่า เหตุใดผู้บริโภคจึงชื่นชอบในการรับประทานเนื้อสุกรคุโรบูตะ

คำสำคัญ: สุกรคุโรบูตะ สารประกอบที่ให้กลิ่น สารระเหย

Introduction

Pork flavor is a very important quality factor, which is a criterion for Thai consumers in buying pork. For several decades, Thai consumers have preferred buying high lean meat, whereas most of the pig companies have been providing three-way crossbred pigs (Large White x Landrace x Duroc, LYD) because of their high prolific and high meat quantity (Glinoubola et al., 2015).

Currently, Thai people consume about 95% of pork produced in Thailand, or about 15 kg/person/year. In this amount, most of the consumed pigs are LYD pigs. However, there is a small segment of the market called niche market, in which consumers prefer to buy high quality meats such as Kobe beef, Wagyu beef and Kurobuta pork. The high-quality pork, namely Kurobuta or black pig, is very famous in Japan. It has been sold in Japan for nearly half a century. Kurobuta pigs originated from the Berkshire county of England but nowadays Kurobuta pigs can be produced and widely accepted by the consumer in many countries. In Japan, it is reported that the price of Kurobuta pork has gone up by 50% compared with the regular (LYD) pork. (Suzuki et al., 2003; Sasaki et al., 2011; Subramaniyan et al., 2016)

In 2010, pork under the name of “CP-Kurobuta pork” was first introduced to the Thai market by Charoen Pokphand Food (CPF) Public Company Limited. CP-Kurobuta pork has been distributed through modern trade channels in Thailand as premium pork. The CP-Kurobuta pork is well appreciated by Thai consumers for its tenderness and taste (CPF, 2012).

However, aroma flavor is another factor contributing to making different pork quality. Therefore, the objective of this study is to determine the aroma compounds in CP-Kurobuta Pork that make the pork unique.

Materials and methods

1. Materials

Two pig breeds including CP-Kurobuta (CPK) pig and a typical three-way crossbred pig (Duroc x Large white x Landrace) (LYD) were used in this study. All pigs were raised in commercial farms (CPF: Charoen Pokphand Food Public Company Limited) in the eastern part of Thailand. Six female pigs per breed were selected at their marketed weight (105-110 kg). The pigs were transported to a standard commercial slaughterhouse in Chachoengsao province. After the animals were slaughtered, the longissimus muscle (loin) of the left side carcasses between 10th and 12th rib were collected at 24-hour post mortem. Six pork samples from six pigs of each breed (for isolation of volatiles) and three pork sample from three pigs of each breed (for sensory evaluation) were divided into approximately 200 grams of steak, sealed in plastic bag, frozen at -25°C and sent to the laboratory of CPF in Bangkok.

Eighteen meat samples in sealed plastic bags were dipped in 25°C water for defrosting and then removed from the bags to cook in the oven at 180°C (Combi Oven, CTP6-10, ALTO-SHAAM, USA) until core temperature of the meat reached 75°C (Channon, D’Souza & Dunshea, 2016).

2. Isolation of volatiles

2.1 Solid phase microextraction (SPME)

Two grams of each cooked muscle sample was placed in a 10 ml vial tube. The tube was heated at 100°C for 30 seconds and at 70°C for 5 minutes. The volatile aroma compounds were absorbed using SPME device with a fused-silica fiber 50/30 μm (DVB/CAR/PDMS) for 5 minutes and analyzed by gas chromatography-mass spectrometry (GC-MS) (HP Model 6890, Agilent Technologies).

2.2 Simultaneous distillation and solvent extraction (SDE)

Two hundred grams of each cooked sample was grinded coarsely (Grinder, MOULINEX, model AW9, France), 200 ml of diethyl ether and 10 μL of internal standard (2-methyl-3-heptanone) was then added and was shook for 1 hour. The extraction was performed in triplicate. The extracted solution was kept in Duran flask at -40°C for 1 hour. The clear solution was collected out of the fat layer, blowing the solution gently with Nitrogen gas until its volume reached 1 ml, kept in brown bottle at -40°C until analysis.

3. Gas chromatography and mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed on a gas chromatography-mass spectrometry (GC-MS; HP Model 6890, Mass Selective Detector, HP 5973, Agilent Technologies). For the SPME analysis, desorption was performed in splitless mode and the MS was detected with no solvent delay. For the SDE concentrates, 1 μL of extracted solution was injected using splitless mode at 18°C, using Helium gas (99.999%) as a carrier at a flowing rate of 2.2 ml/minutes. The incubator

temperature started at 45°C and then rose 10°C/min to 220°C, hold at 220°C for 65 minutes. Two capillary columns were connected with the retention gap including HP-5 column and DB-wax column (60 m long, 250 μm in diameter and 0.25 μm of film thickness). A series of n-alkanes (C5-C25) were operated under the same conditions to obtain the retention index (RI) values for the aroma compounds. The MS was operated in electron impact mode with an electronic energy of 70 eV, a scanning range of 30-300 m/z and speed 2.74 scan/sec.

Aroma compounds were identified by comparing their mass spectra with those contained in the Wiley 275 library databases and their retention indexes (RI) were compared with the RI of standard for n-alkane (C5-C30) as well. Approximate quantities of the aroma compounds were estimated by comparison of their peak areas with that of the 2-methyl-3-heptanone internal standard using Chemstation software B.02.05 (Agilent Technologies).

4. Gas chromatography and olfactometry (GC-O) analysis

The extracted solutions from CPK pork and LYD pork were conducted in Aroma Extract Dilution Analysis (AEDA) to determine the relative potency of individual odorants. Stepwise dilutions (1:3, 1:9 and 1:27) were prepared with diethyl ether. Two trained assessors tested the odor of the volatile compounds using Gas Chromatography-Olfactometry (GC-O). The column was split into two ports (ratio 1:1); the first port connected to a Flame Ionization Detector (FID) at 220°C, and the second port connected to sniffing port. The type of columns and the condition of GC-MS have

been previously described. Panelists tested the aroma compounds at the sniffing port and then compared their RI for indicating type of the odorants and reported a Flavor Dilution (FD) factor of each compound.

Sensory evaluation

Twelve trained panelists evaluated the cooked pork samples. Each sample was assessed within 3 minutes after cooking using the scoring scale that was 15 centimeters long for describing the aroma odor. The evaluations were performed in 3 replicates.

Statistical analysis

The amounts of extracted compounds were the averages of six replicates. The sensory panel scores were the averages of three replicates. All the variants were analyzed using SPSS software (version 10.5). The significant differences between means at the confidence level 95% were performed using Duncan's Multiple's Range Test (DMRT) method.

Results and discussions

In this study, volatiles were isolated by both SPME and SDE method. The results showed that the SDE method provided a more complete number of aromatic volatiles and the quantitative data.

From table 1 and 2, 47 flavor compounds were detected in CPK pork whereas only 20 flavor compounds were identified in LYD pork. These flavor compounds were divided into 7 types, including aldehydes, ketones, amines, acids, alcohols, alkanes and alkenes.

We found 8 aldehydes, 3 ketones, 9 amines, 7 acids, 5 alcohols, 6 alkanes and 9 alkenes in CPK pork, and 5 aldehydes, 1 amine, 1 acid, 4 alkanes and 9 amines were detected in LYD pork. Some flavor compounds (ketones and alcohols) were not detected in LYD pork.

For the relative content of common compounds in two pork groups, some compounds had significant differences in amount among these two groups. The content of 5 aldehydes (hexanal, benzylaldehyde, cinamaldehyde, nonanal and decanal), 1 amine (2-aziridin ethylamine), 1 acid (acetic acid), 2 alkanes (pentane and hexane) and 6 alkenes (hexene, octene, nonene, undecene, toluene and limonene) in CPK pork were significantly higher than LYD pork ($P < 0.05$), while the content of 1 alkane (Heptane) and 1 alkene (Dodecene) in LYD were significantly higher than CPK pork ($P < 0.05$). The GC-MS results showed that we could determine the differences of volatile compounds between two different pig breeds. Jian et al. (2014) studied the volatile flavor compounds of cooked meats from four different pig breeds including lean-type pig and three other Chinese pig breeds (Wannan spotted pig, Anqing six-white-spotted pig and Dinguyan black pig) and found that there were many volatiles correlated with pork aroma in Chinese pig breeds such as Limonene, hexanal, heptanal, (E)-tetradecene-1-ol, 2-pentylfuran, 2-ethylfuran, 2-hexylfuran, 14-octadecenal, heptanone, Benzaldehyde, undecanal, octanal, and (E)-2-tetradecene-1-ol. However, no volatile compounds were correlated with the meat aroma of the lean-type pig.

Table 1 Volatiles identified in GC-MS by both SPME and SDE from the roasted pork of CP-Kurobuta (CPK) pigs / Landrace x Yorkshire x Duroc (LYD) crossbred pigs

Compounds	SPME		SDE				RI ²	
	Peak area (%)		Peak area (%)		Amount (ng/g) ¹		HP5	DBWAX
	CPK	LYD	CPK	LYD	CPK	LYD		
<i>Aldehyde Group</i>								
Butanal	0.64±0.03	-	0.76±0.08	-	6.33±0.94	-	<600	862
Pentanal	0.83±0.02	-	0.95±0.14	-	8.03±0.21	-	623	921
Hexanal	5.23±0.08	-	2.43±0.17	0.02±0.03	38.66±0.42 ^a	0.85±0.02 ^b	799	1072
Benzylaldehyde	0.29±0.04	-	23.27±0.23	0.12±0.03	168.43±0.22 ^a	0.99±0.13 ^b	946	1514
Cinamal aldehyde	17.68±0.23	-	21.04±0.12	0.07±0.03	143.35±0.11 ^a	1.05±0.16 ^b	1002	1735
Nonanal	0.55±0.07	-	19.52±0.07	0.06±0.04	99.67±0.24 ^a	1.08±0.09 ^b	1104	1379
Decanal	0.43±0.09	-	1.32±0.93	0.17±0.02	9.42±0.17 ^a	0.95±0.12 ^b	1204	-
Undecanal	0.09±0.02	-	1.57±0.06	-	8.72±0.10	-	1263	-
<i>Ketone Group</i>								
4,6-Hepta-3-one	0.53±0.07	-	0.56±0.08	-	5.90±0.02	-	678	978
1-Pentanone	-	-	0.04±0.02	-	8.79±0.31	-	758	-
2-Nonanone	-	-	0.02±0.01	-	4.08±0.16	-	1087	1564
<i>Amine Group</i>								
2-Aziridin ethylamine	23.25±0.42	9.87±0.23	43.59±0.13	7.65±0.04	248.42±0.68 ^a	13.19±0.22 ^b	<600	<600
1,2-Propanediamine	9.63±0.82	-	10.42±0.76	-	73.43±0.54	-	1032	1734
Propylcyclo Hexylamine	-	-	1.43±0.02	-	8.72±0.10	-	10.87	1785
Hydrocy benzylamine	-	-	5.79±0.17	-	13.48±0.16	-	12.43	1938
Thiophenyl-2-methylamine	-	-	0.22±0.01	-	2.43±0.02	-	1328	-
Benzaoxanamine	10.42±0.09	-	12.64±0.16	-	23.21±0.15	-	1368	1890
Methylphenyl benzamie	-	-	0.62±0.05	-	5.90±0.02	-	1352	1925
Propanamide	-	-	0.47±0.04	-	5.74±0.11	-	1389	1932
Benzylphenethylamine	-	-	0.03±0.02	-	4.21±0.19	-	1405	1928

¹ Means and standard errors expressed as ng/g sample, means derived from six replicate samples.

² Retention index (RI), agrees with retention index of the database or agrees with retention index and mass spectrum of standard chemical. RI are given on two different polarity capillary columns, the nonpolar column (HP-5) and the polar column (DBWAX).

Moreover, we also analyzed the top three aroma contents in each pork group, we found that aldehydes (482.61 ng/g), amines (385.54 ng/g) and alkenes (374.09 ng/g) were the three highest contents in CPK pork, while the top three aroma contents in LYD pork were alkenes (226.73 ng/g), alkanes (76.76 ng/g) and amines (13.19 ng/g).

In this study, the aroma active compounds of pork were extracted by the SDE method.

The extract was diluted according to the volume ratios. The diluted sample was injected for the GC-O analysis until the sniffers at the GC-O terminal could not detect the smell, in which the higher dilution of a compound suggests its higher level of contribution to the overall aroma. The highest obtained dilution ratio was defined as the FD factor.

From table 3 and 4, CPK pork had higher number of sniffed aroma active compounds (36 compounds) than LYD pork (9 compounds). From previous studies, 43 aroma active com-

pounds were detected in the roasted pork of Mini-pig (Xie et al., 2008) and 16 aroma active compounds were detected in the cooked cured pork ham (Benet et al., 2016).

Table 2 Volatiles identified in GC–MS by both SPME and SDE from the roasted pork of CP-Kurobuta (CPK) pigs / Landrace x Yorkshire x Duroc (LYD) crossbred pigs (continued)

Compounds	SPME		SDE				RI ²	
	Peak area (%)		Peak area (%)		Amount (ng/g) ¹		HP5	DBWAX
	CPK	LYD	CPK	LYD	CPK	LYD		
Acid Group								
Acetic acid	4.32±0.04	0.97±0.06	5.49±0.04	1.23±0.62	4.32±0.04 ^a	0.97±0.06 ^b	704	1117
Butanoic acid	0.79±0.04	-	0.04±0.00	-	0.79±0.04	-	752	1612
Pentanoic acid	8.13±0.94	-	0.93±0.12	-	38.13±0.94	-	789	1829
Cabamic acid	4.56±0.02	-	0.02±0.01	-	4.56±0.02	-	835	1762
Sulfuric acid	3.69±0.03	-	0.02±0.01	-	3.69±0.03	-	876	1854
Formic acid	196.87±0.18	-	7.83±0.02	-	96.87±0.18	-	899	1428
Propanoic acid	60.78±0.23	-	2.43±0.02	-	60.78±0.23	-	976	1433
Alcohol Group								
3-Pentanol	0.82±0.03	-	0.04±0.00	-	0.82±0.03	-	632	1204
2-ethyl-1-hexanol	2.71±0.02	-	0.03±0.01	-	2.71±0.02	-	672	1274
hexanol	2.78±0.04	-	0.03±0.01	-	2.78±0.04	-	723	1302
Metanol	5.63±0.06	-	0.04±0.02	-	5.63±0.06	-	764	1355
Octanol	0.41±0.02	-	0.01±0.00	-	0.41±0.02	-	823	1402
Alkane Group								
Pentane	-	-	2.39±0.05	2.02±0.04	50.83±0.25 ^a	48.21±0.39 ^b	720	720
Hexane	-	-	0.04±0.02	0.03±0.01	3.61±0.02 ^a	2.43±0.64 ^b	780	780
Heptane	-	-	0.04±0.03	0.07±0.03	3.23±0.04 ^b	8.69±0.43 ^a	825	825
Cyclohexane	-	-	0.09±0.07	0.09±0.04	17.28±0.06	17.43±0.64	840	840
Cyclooctane	-	-	6.43±0.04	-	188.61±0.43	-	890	890
Silane	-	-	0.04±0.09	-	3.54±0.07	-	930	930
Alkene Group								
Benzene	-	-	0.09±0.03	0.09±0.04	18.02±0.13	18.07±0.19	752	813
Hexene	-	-	0.21±0.05	0.06±0.04	22.13±0.17 ^a	12.29±0.45 ^b	782	826
Heptene	-	-	0.09±0.01	0.09±0.02	18.26±0.48	18.22±0.34	823	925
Octene	-	-	0.10±0.03	0.05±0.03	18.26±0.42 ^a	14.26±0.41 ^b	852	1029
Nonene	-	-	3.29±0.07	2.43±0.06	66.36±0.53 ^a	35.68±0.68 ^b	902	1176
Undecene	-	-	4.52±0.04	2.38±0.04	74.65±0.43 ^a	35.68±0.61 ^b	992	1484
Dodecene	-	-	2.14±0.02	3.02±0.04	38.40±0.60 ^b	43.27±0.82 ^a	1091	1689
Toluene	-	-	3.24±0.02	3.20±0.07	53.62±0.83 ^a	47.82±0.45 ^b	1013	1708
Limonene	-	-	4.25±0.07	0.02±0.01	64.39±0.42 ^a	1.44±0.23 ^b	1132	1762

¹ Means and standard errors expressed as ng/g sample, means derived from six replicate samples.

² Retention index (RI), agrees with retention index of the database or agrees with retention index and mass spectrum of standard chemical. RI are given on two different polarity capillary columns, the nonpolar column (HP-5) and the polar column (DBWAX).

Table 3 GC–O analysis of volatiles isolated by SDE from the roasted pork of CP-Kurobuta (CPK) pigs / Landrace x Yorkshire x Duroc (LYD) crossbred pigs.

Compounds	RI ¹		Odor descriptions	Log ₃ FD factor ²	
	HP5	DBWAX		CPK	LYD
<i>Aldehyde Group</i>					
Butanal	<600	862	sweet, sweet roasted pork, oil	0	-
Pentanal	623	921	sweet roasted pork	0	-
Hexanal	799	1072	sweet roasted pork	0	0
Benzyl aldehyde	946	1514	roasted pork, almond, smoke	1	0
Cinamaldehyde	1002	1735	cinnamon	1	0
Nonanal	1104	1379	sweet roasted pork, honey, citrus	1	0
Decanal	1204	-	sweet roasted pork, kaffir lime, oil, honey	0	0
Undecanal	1263	-	sweet roasted pork, oil, caramel, citrus	0	-
<i>Ketone Group</i>					
4,6-hepta-3-one	678	978	sweet caramel, sweet flower	0	-
1-Pentanone	758	-	sweet roasted pork	0	-
2-Nonanone	1087	1564	sweet roasted pork	0	-
<i>Amine Group</i>					
2-Aziridin ethylamine	<600	<600	sweet roasted pork, honey	2	1
1,2-Propanediamine	1032	1734	sweet roasted pork	1	-
Propylcyclo hexylamine	1087	1785	roasted pork	0	-
Hydrocyl benzylamine	1243	1938	roasted pork	1	-
Thiophen-2-methylamine	1328	1879	roasted pork	0	-
Benzaoxanamine	1368	1890	roasted pork	0	-
Methylphenyl benzamine	19215	1352	roasted pork	0	-
Propanamide	1389	1932	roasted pork	0	-
Benzylphenethylamine	1405	1928	roasted pork	0	-

¹ Retention index (RI), agrees with retention index of the database or agrees with retention index and mass spectrum of standard chemical. RI are given on two different polarity capillary columns, the nonpolar column (HP-5) and the polar column (DBWAX).

² Log₃ FD factor = 0 mean detected odor before diluted aroma extract for Aroma Extract Dilution Analysis (AEDA), = 1 mean detected odor after dilution 1:3, = 2 mean detected odor after dilution 1:9, and = - mean no detected odor.

In this study, the 36 determined aroma active compounds in CPK pork consisted of 8 aldehydes, 3 ketones, 9 amines, 7 acids, 5 alcohols, 2 alkanes and 2 alkenes, while in LYD pork the 9 determined aroma active compounds consisted of 5 aldehydes, 1 amine,

1 acid and 2 alkenes. 6 out of 36 aroma active compounds of CPK pork were potent aroma compounds (Log₃ FD factor >0) including 2-Aziridin ethylamine (sweet roasted pork, honey) (Log₃ FD factor = 2), benzyl aldehyde (roasted pork, almond, smoke), cinamaldehyde

(cinnamon) and nonanal (sweet roasted pork, honey, citrus), 1,2-Propanediamine, hydrocyl benzylamine (roasted pork) (Log₃ FD factor = 1). In LYD pork, 2-Aziridin ethylamine (sweet roasted pork, honey) (Log₃ FD factor = 1) was the only potent aroma compound out of the 9 total aroma active compounds (table 3 and 4).

The GC-O results in this study revealed the unique aroma active compounds of CPK pork and LYD pork. According to the aroma profile

of the potent odorants, the CPK pork shows the more desirable sweet honey-like flavor and more intensity of roasted pork flavor. Recently, Zhao et al. (2017) studied the volatile compounds in the stewed pork broth (from black-pig and the common white pig) and found that most of the aroma active compounds found were nearly the same but with different FD values. However, the black-pig broth shows the more desirable meaty flavor and more roasted notes.

Table 4 GC–O analysis of volatiles isolated by SDE from the roasted pork of CP-Kurobuta (CPK) pigs / Landrace x Yorkshire x Duroc (LYD) crossbred pigs (continued).

Compounds	RI		Odor descriptions	Log ₃ FD factor	
	HP5	DBWAX		CPK	LYD
Acid Group					
Acetic acid	704	1117	vinegar, sweet	0	0
Butanoic acid	752	1612	sweet, flower	0	-
Pentanoic acid	789	1829	sweet, flower	0	-
Cabamic acid	835	1762	sweet, flower	0	-
Sulfurous	876	1854	sweet, flower, fruit	0	-
Formic acid	899	1428	formic	0	-
Propanoic	976	1433	sweet, flower	0	-
Alcohol Group					
3-Pentanol	623	1204	sweet fruit	0	-
2-ethyl-1-hexanol	672	1274	dried shredded pork	0	-
Hexanol	723	1302	dried shredded pork	0	-
Metanol	764	1355	alcohol	0	-
Octanol	823	1402	sweet fruit	0	-
Alkane Group					
Cyclooctaxane	890	890	newspaper	0	-
Silane	930	930	roasted pork	0	-
Alkene Group					
Toluene	1013	1708	smoke	0	0
Limonene	1132	1762	Bergamot, orange, sweet	0	0

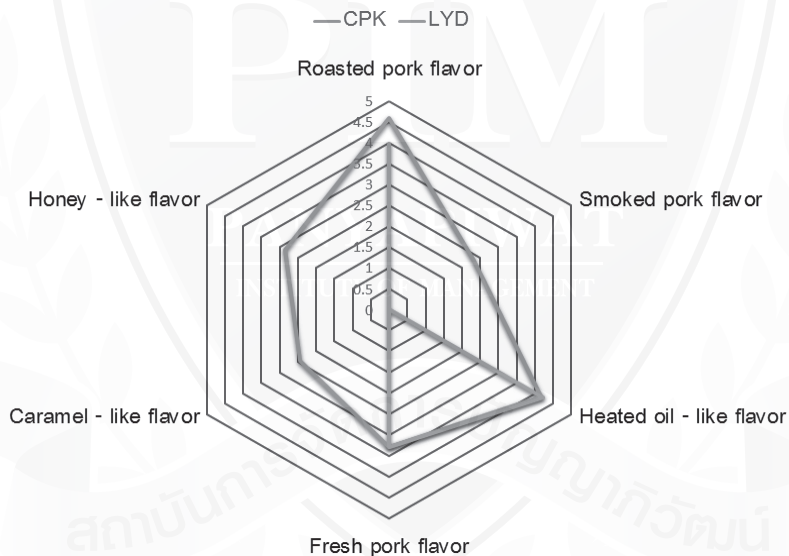
¹ Retention index (RI), agrees with retention index of the database or agrees with retention index and mass spectrum of standard chemical. RI are given on two different polarity capillary columns, the nonpolar column (HP-5) and the polar column (DBWAX).

² Log₃ FD factor = 0 mean detected odor before diluted aroma extract for Aroma Extract Dilution Analysis (AEDA), = 1 mean detected odor after dilution 1:3, = 2 mean detected odor after dilution 1:9, and = - mean no detected odor.

Table 5 Sensory evaluation of the roasted pork of CP-Kurobuta (CPK) pigs / Landrace x Yorkshire x Duroc (LYD) crossbred pigs

Flavor characteristics	Panelists Score (1-5)*	
	CPK	LYD
Roasted pork flavor	4.58±0.43	3.98±0.26
Smoked pork flavor	2.34±0.12	-
Heated oil - like flavor	4.16±0.98	4.23±0.42
Fresh pork flavor	3.28±0.76	3.30±0.15
Caramel - like flavor	2.43±0.78	-
Honey - like flavor	2.86±0.23	-

* Flavor attributes were scored by a 5-point scale where 1 = extremely weak, to 5 = extremely strong.

**Figure 1** Spider-web for the top 6 odorants in the cooked pork of the CPK/LYD.

The full distance on the scale was defined to be 5

The results of sensory analysis (Table 5 and Fig.1) corresponded with the results of volatile compounds analysis. Sensory analysis showed that cooked pork from CPK pork had higher number of detected pork flavors than LYD pork. Panelists detected 6 flavors (roasted

pork, smoked pork, heated oil, fresh pork, caramel and honey) in cooked CPK pork, while only 3 flavors (roasted pork, heated oil, fresh pork) were detected in LYD pork. For those detected flavors, “roasted pork flavor” in CPK pork had higher intensity score than in LYD.

This result agreed with the study of Wood et al. (2004), comparing the sensory properties of cooked meat samples from two traditional (Berkshire, Tamworth) and two modern (Duroc, Large White) pig breeds. The traditional breeds had higher intramuscular fat content in the meat, and were higher in pork flavor and desirable flavor than the modern breeds.

The results in this study indicated that cooked pork of CPK pig is higher in both number and the concentration of the volatiles than LYD pig. This could be the differences in microstructure or aroma precursor profiles (fatty acids, amino acids) of the CPK pork compared with LYD pork. Many publications agree that the genetic background of pigs plays an important role in the eating quality of pork due at least in part to genetic effects on the muscle microstructure (Lee, Kang & Kang, 2006; Sorapukdee et al., 2013; Glinoubol et al., 2015). Subramaniyan et al. (2016) reported that meat from Berkshire (Kurobuta) pigs had higher levels of amino acids (phosphoserine, aspartic acid, threonine, serine, asparagine, α -aminoadipic acid, valine, methionine, isoleucine, leucine, tyrosine, histidine, tryptophan, and carnosine) and lower levels of glutamic acid, glycine, alanine, and ammonia than that meat from LYD pigs. The fatty acids oleic acid, docosahexaenoic acid (DHA), and monounsaturated fatty acids (MUFA) were present in significantly higher concentrations in Kurobuta pork than those in LYD pork.

Conclusions

Distinct differences in aroma active compounds of longissimus muscle from CPK pigs and LYD pigs were observed in this study. 47 volatile compounds in the pork of CPK pigs were identified by GC-MS with the dominant amounts of aldehydes, amines and alkenes, while only 20 volatile compounds were detected in LYD pork with the dominant amounts of alkenes, alkanes and amines. 36 aroma active compounds were determined in CPK pork by ADEA-GC-O, while only 9 aroma active compounds were detected in LYD pork. 6 out of 36 aroma active compounds of CPK pork were potent aroma compounds including 2-Aziridin ethylamine (sweet roasted pork, honey), benzyl aldehyde (roasted pork, almond, smoke), cinamaldehyde (cinnamon), nonanal (sweet roasted pork, honey, citrus), 1,2-Propanediamine, hydrocyl benzylamine (roasted pork). In LYD pork, 2-Aziridin ethylamine was the only potent aroma compound. The results of sensory analysis corresponded with the results of volatile compounds analysis. The unique aroma flavor of roasted CPK pork and LYD pork are revealed for the first time. These results could be one of the reasons why consumers prefer the Kurobuta pork.

Acknowledgements

The present work was funded by Panyapiwat Institute of Management (PIM) and supported by the Pig Business of CPF - Charoen Pokphand Food Public Company Limited, Thailand.

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