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Changes in volatile compounds during fermentation of nham (Thai fermented sausage)

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Abstract: Nham samples fermented by indigenous microorganisms and *Lactobacillus curvatus* starter culture were studied for their compositions of volatiles in comparison to a non-fermented sample which was prepared by adding antibiotics to the ingredients. The incubation for all samples was at 30 °C for 3 days. Volatile compounds of nham were isolated and analysed using dynamic headspace sampling–gas chromatography-mass spectrometry (DHS-GC-MS). Fermentation of nham resulted in a decrease in aldehydes and sharp increases in esters and alcohols in the first day. Natural fermented nham and nham inoculated with 10^4 cfu/g *L. curvatus* starter culture (LC) were similar in their concentrations of total aldehydes, alcohols, esters and sulphur compounds. The most prominent aroma components identified by their odour active values (OAV) were ethyl butanoate (*buttery, ripe fruit note*) and sulphur-containing compounds from garlic. Other aroma impact compounds were ethyl 2-methylbutanoate (*fruity*), ethyl 2-methylpropanoate (*fruity*), 3-hydroxy-2-butanone (*yoghurt-like*), octanal (*fatty-fruity*) and hexanal (*green*). Ethyl butanoate, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate and octanal were from microbial activity in the fermented samples whereas hexanal and 3-hydroxy-2-butanone were also found in the non-fermented sample. The intensities of "fermented", "acidic", "garlic" and "overall flavour of nham" aroma evaluated by ten trained panelists, increased sharply on the first day. Acidic, garlic and overall flavour of nham aroma intensities then remained almost constant. The intensity of fermented aroma in LC nham, however, continued to increase on the second day. This was concomitant with the increase in ethyl butanoate (OAV = 9072). In comparison to the natural fermented sample, LC nham had a faster reduction in pH, higher fermented aroma on the second day, and higher concentrations of ethyl butanoate and 3-hydroxy-2-butanone.

Keywords: Nham, fermented sausage, *Lactobacillus curvatus*, volatile compounds, ethyl butanoate

Introduction

Nham is a fermented sausage commonly consumed in Thailand and Southeast Asia. It is prepared from ground pork and pork rinds mixed with cooked rice, Thai chili, garlic, and other additives, i.e., nitrite, phosphate, salt and sugar. Fermentation in nham is usually accomplished by natural microflora within 3-5 days at 30 °C. The sausage is generally consumed uncooked approximately three

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days after production. Unlike European dry fermented sausages, nham is prepared without the ripening stage during the manufacturing process. The predominant microorganisms in nham are lactobacilli and pediococci (Tanasupawat and Komagata, 1995). The common lactobacillus strains in commercial nham are *L. acidophilus, L. cellobiose, L. plantarum, L. pentosus, L. curvatus, L. saki, L. delbrückii, L. paracasei and L. brevis* (Valyasevi *et al.*,1999). Other microorganisms identified at the early stage of fermentation include *Micrococcus varians*, yeasts and moulds.

Aroma compounds in dry fermented sausages are derived from seasonings, lipid oxidation and the microbial metabolism of lipids, proteins and carbohydrates. The composition of volatile compounds is dependent on the types of meat, fat, nitrate, nitrite, salt and spice used as well as processing conditions (Sunesen *et al.*, 2001; Olesen *et al.*, 2004; Marco *et al.*, 2008). The sausage flavour in dry fermented sausage depends on the concentrations of several ethyl esters, methyl ketones, and 2- and 3-methyl butanal produced by the microorganisms (Stahnke, 1995). 2-Methyl-propanoic acid, and 2- and 3-methylbutanoic acids cause rancid and cheesy notes in dry sausage (Stahnke, 1995). Lipid oxidation leads to the formation of some alkanes, alkenes, methyl ketones, aldehydes, and alcohols (Marco *et al.*, 2008, Berdagué *et al.*, 1993).

Starter cultures have been used in manufacturing of fermented sausages to ensure lactic acid production, enhance flavour and improve product safety. There have been several investigations on aroma compounds of European dry fermented sausages produced by starter cultures such as *Staphylococcus xylosus* (Stahnke, 1994), *S. carnosus* (Olesen *et al.*, 2004), *Penicillium aurantiogriseum* (Bruna *et al.*, 2001), *Micrococcus varians*, *P. nalgiovense*, *L. curvatus*, and *Debaryomyces* spp. (Flores *et al.*, 2004) . Khieokhachee *et al.* (1997) used pure cultures of *L. curvatus, L. sakei or L. plantarum* and found that the product using *L. curvatus* gave the best overall acceptability scores in sensory evaluations. The level of inoculum also plays a role in aroma quality. Nham inoculated with 10^4 cfu/g *L. curvatus* had better flavour than the natural fermented sample (Visessanguan *et al.*, 2006).

The aroma is of great importance to nham quality, but little is known about the components that comprise its aroma profile. The purpose of this research was to investigate the changes in volatile compounds in natural fermented nham and nham produced by using *L. curvatus* as starter culture.

Materials and Methods

In this experiment nham fermented by indigenous microorganisms and nham inoculated with pure culture of *L. curvatus* were studied. Pure culture of *L. curvatus* was obtained from BIOTEC Culture Collection, Thailand. A non-fermented sample was prepared to observed changes in volatile compounds in the nham mixture without the interference of microbial fermentation. All experiments were done in duplicate.

Preparation of nham

Three separate batches of nham (N), nham inoculated with pure culture of *L. curvatus* (LC), and non-fermented nham (NF) mixture were prepared. The meat portion of nham consisted of 60% ground pork and 40% shredded cooked pork rinds. The weights of the other ingredients were calculated as percentages of the meat portion weight. Minced cooked rice (5/100 g), minced fresh garlic (5/100 g), sodium chloride (2/100 g), whole bird chili (1/100

g), sucrose (0.5/100 g), monosodium glutamate (0.2/100 g), sodium tripolyphosphate (0.2/100 g), sodium erythorbate (0.2/100 g) and 125 ppm sodium nitrite (0.0125/100 g) were mixed thoroughly with the pork and pork rinds. LC nham was prepared in a similar manner to the natural fermented sample but was inoculated with *L. curvatus* starter culture (BIOTEC, Pathumthani, Thailand). The culture was made into a suspension and inoculated nham at the dose of 10^4 cfu/g. All mixtures were packed tightly in 5 cm diameter x 6 cm high glass jars and covered with aluminium foil and parafilm. The fermentation was carried out in a multi-thermo incubator (Rexall Industries Co., Taiwan) at 30°C for 3 days. Samples were collected at days 0, 1, 2 and 3 of fermentation and wrapped in aluminium foil, vacuum packed in polyethylene bags and stored at -40°C. The frozen samples were brought to room temperature before analyses.

The non-fermented sample was prepared using the method described by Stahnke (1994). Nham ingredients were added with 2g/kg potassium sorbate and antibiotics, that were 100 mg/kg chloramphenicol (Sigma-Aldrich Co., St. Louis, MO), and 100 mg/kg chlortetracycline hydrochloride (Sigma-Aldrich Co., St. Louis, MO).

pH measurement

Five grams of sample was homogenised with 10 ml of distilled water (AOAC, 2000). The pH was determined using a standard pH meter (Omega PHB-62; Omega, Taiwan).

Microbiological analyses

Samples were evaluated for changes in numbers of lactic acid bacteria and total plate counts (TPC). Ten grams of sample from each batch was removed aseptically, and added to 90 ml of 0.1% sterile peptone water in a sterile stomacher bag. The samples were homogenised in a stomacher lab blender (Seward model 400; Seward, London, UK) for 2 min. Serial dilutions were made with 0.1% peptone water. Samples were evaluated for the numbers of lactic acid bacteria (LAB) during fermentation by plating ten-fold dilutions onto lactic acid bacteria selective media, deMann-Rogosa-Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany), and overlaying with the same media. The incubation was performed under anaerobic condition at 35°C for 2 days.

Total bacterial growth was determined on plate count agar (PCA; Scharlau, Spain). The plates were incubated at 35°C for 2 days. The results were reported as log cfu/g.

Volatile compounds analysis

Fifteen grams of nham was ground in a Waring blender. Ground nham (1.00 g) was removed and placed into a 40-ml purge vial. Ten microlitres of the internal standard solution (4.28 mg/5 ml of 2-methyl-3-heptanone in methanol) was added to the vial. A purge-and-trap concentrator (Tekmar-3000, Teledyne Technologies Inc., Mason, OH, USA) was used to collect volatiles from the sample. The sample was purged at 35 °C with helium (99.99%) at a rate of 40 ml/min for 30 min. Volatile compounds were trapped in a Tenax TA trap (Teledyne Technologies Inc., Mason, OH, USA) at 35°C. The trap was then thermally desorbed by heating at 200°C for 3 min and back-flushed through the transfer line to the capillary interface. The back-flushed volatiles were cryofocused at -100°C with liquid nitrogen and then rapidly heated to 200°C and injected into an HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). Additional parameters in the order of automation sequence were:

prepurge for 2 min at 35°C, preheat for 5 min at 50°C, dry purge for 1 min, desorption preheat to 195°C, and bake for 20 min at 200 °C.

The volatiles were analysed using an HP 6890 GC system coupled to an HP 5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The separation was performed on a 5% phenyl-methyl silicone bonded phase fused silica capillary column (HP-5, 60 m x 0.25 m id x 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA). The oven program was: 30°C for 2 min, 2°C/min to 60°C, 10°C/min to 100 °C, 20 °C/min to 140°C, 10°C/min to 200°C, and hold at 200°C for 10 min. The inlet temperature was set at 200°C. Helium was used as carrier gas at a flow rate of 1.1 ml/min. The detector was operated in the scan frequency of 2.74 scans/s with a scan range of 30-300 amu and the ionisation energy was 70 eV. The ion source was held at 230°C.

Identification of volatiles was achieved by comparing mass spectral data with those of the Wiley 275 mass spectral library and the retention indices with those of standard compounds and information from the literature.

Sensory evaluation

The sensory evaluation method used in this work was adapted from the method described by Stanke (1995). The odour of nham was evaluated by ten trained panelists. The quantitative descriptive method was used with the descriptors and an unstructured line scale of 150 mm. The anchor points indicating the lowest and highest levels were marked at 15 mm from both ends of the line. The list of descriptors was developed by the panel members during the training sessions. The test samples consisted of approximately 6 g of ground nham equilibrated in closed 65 ml glass containers for 30 min at 37°C. The panel was presented with a reference sample at the beginning of each session.

Statistical analysis

ANOVA was used to indicate the significant differences between mean values of the different results. A comparison between samples and fermentation time was performed using Duncan's multiple range test. Significant level was established at $P < 0.05$.

Results and Discussion

pH value

Changes in the pH of nham samples during fermentation are given in Figure 1. At the beginning of the fermentation, samples had pH values between 6.14 and 6.37. During incubation, the pH of both fermented samples decreased due to lactic acid generated by lactic acid bacteria. After 3 days, LC nham had the lowest pH value of 4.17 followed by the natural fermented nham that had a pH of 4.59. The pH of non-fermented nham remained constant at above 6 throughout the experiment. Nham is usually consumed when the pH drops to 4.4-4.6 (Phitakpol *et al.*, 1995). LC nham was ready to be consumed after 2 days of fermentation whereas the sample fermented by indigenous microorganism required 3 days to reach the pH necessary for acceptable eating quality.

Microbial changes

Changes in TPC are given in Figure 2. The TPC of both LC nham and the natural fermented sample increased during fermentation. At the beginning of fermentation, LC nham had the highest TPC and the number of LAB because of the inoculation of *L. curvatus*. The viability of LAB in LC nham and the natural fermented sample reached the highest level during day

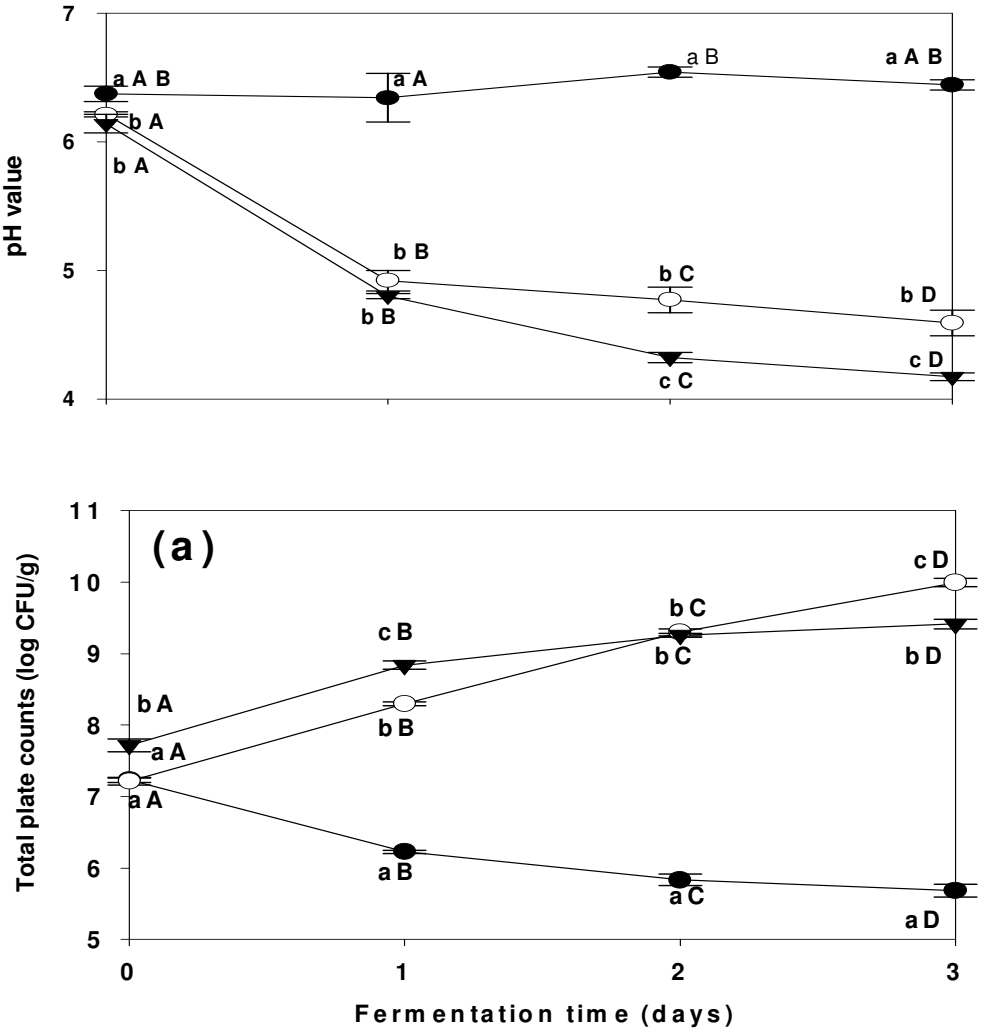

Figure 1. Changes of pH during fermentation in nham with added antibiotics (●), natural fermented nham (○) and nham fermented with *L. curvatus* (▼). a-c: Different letters on the same day are significantly different ($P < 0.05$). A-D: Different letters in the same nham sample are significantly different ($P < 0.05$)

2 and then decreased slightly on day 3 (Figure 2). After 2 days of fermentation, the number of LAB in LC nham was significantly lower ($P < 0.05$) than that of the natural fermented nham. This is possibly because *L. curvatus* generated lactic acid at a faster rate than the indigenous LAB resulting in a faster drop in pH that decreased the viability of microorganism.

In the non-fermented sample, antibiotics efficiently inhibited the growth of microorganisms but did not immediately kill the cells. The numbers of microorganisms gradually decreased through the incubation period.

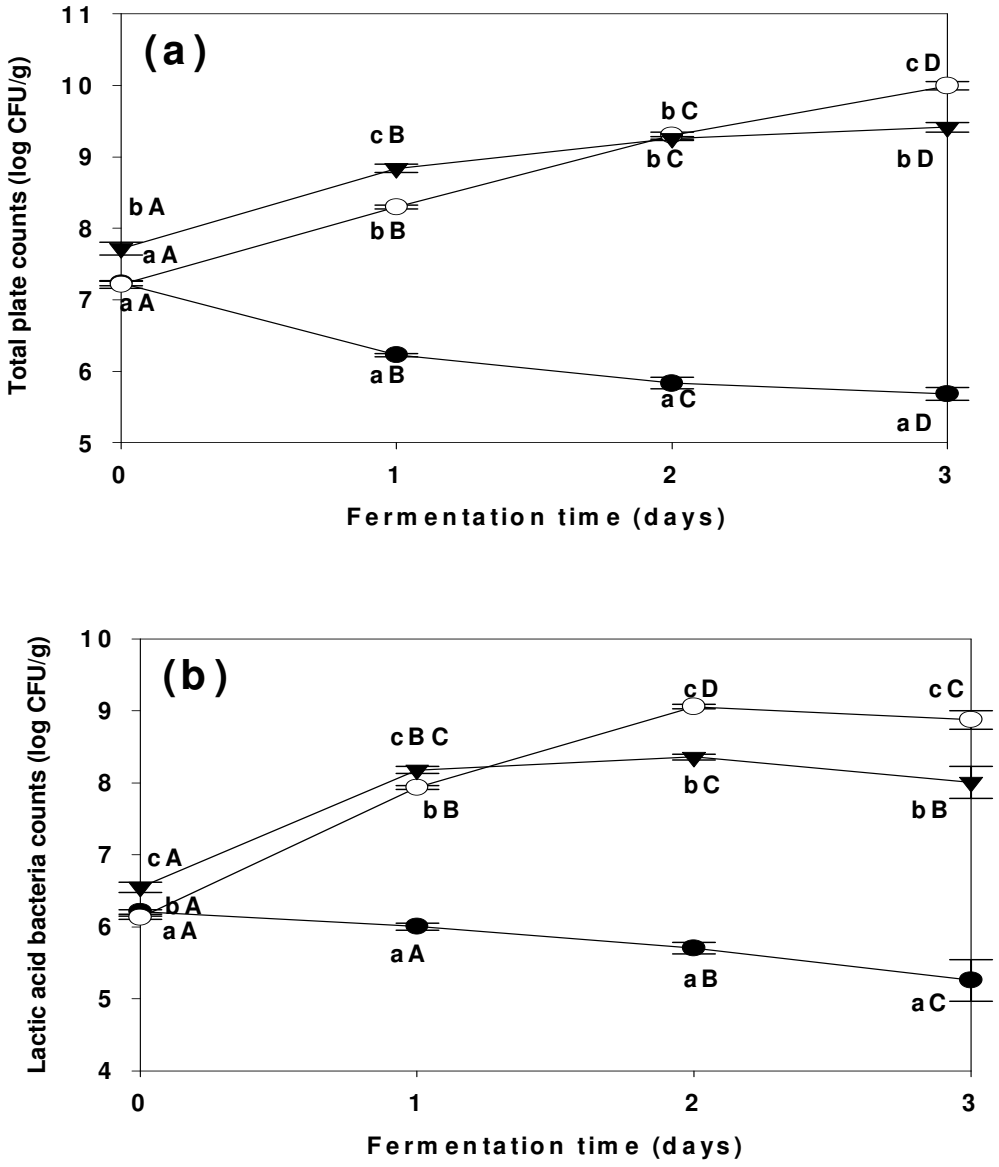

Figure 2. Changes in (a) total plate counts and (b) lactic acid bacteria counts during fermentation of nham with added antibiotics (●), natural fermented nham (○) and nham fermented with *L. curvatus* (▼). a-c: Different letters on the same day are significantly different ($P < 0.05$). A-D: Different letters in the same nham sample are significantly different ($P < 0.05$)

Volatile compound analysis

Changes in volatile compounds in fermented samples are shown in Table 1. Table 2 shows only compounds that changed in the non-fermented sample during the 3-day incubation. In the non-fermented sample, many volatile compounds, especially esters were not found and several compounds remained constant during the period. A total of 113 volatile compounds were identified in the three samples. These included 9 hydrocarbons, 12 aldehydes, 9

ketones, 19 alcohols, 25 esters, 29 sulphur compounds, 2 acids, 1 terpene, 1 furan, 2 halogens and 4 miscellaneous compounds. The most prominent odourants in nham were sulphur compounds, alcohols and esters that were different from aldehydes and acids in European dry fermented sausage reported by Marco *et al.* (2008).

Sulphur compounds were the most abundant compounds in all samples due to the use of garlic in the formulation. The compound presented in the highest concentration was di-2-propenyl disulfide followed by 3,3′-thiobis-1-propene. The concentration of sulphur-containing compounds in all samples increased considerably during the first day of fermentation and then remained almost constant. Changes in sulphur compounds in fermented samples (Table 1) and non-fermented sample (Table 2) were similar. This indicates that most changes in sulphur compounds were not dependant on microbial metabolism. The increase of sulphur compounds could be due to the conversion of allicin by allinase to various odoriferous compounds and their subsequent transformation. Allinase activity starts immediately after the damage of garlic parenchyma. These reactions presumably continued in the sausage during processing and storage (Mateo and Zumalacárregui, 1996).

Alcohols were the second most abundant volatiles in nham. Total alcohol concentration was at the same level for all samples at the beginning ($P > 0.05$). A remarkable increase in alcohols, especially ethanol, during the first day of fermentation was observed in all samples including the non-fermented sample. This indicates that ethanol and other alcohols can form in the absence of microbial activity. However, the presence of microorganism resulted in higher concentrations of alcohols in the fermented than the non-fermented samples.

In the non-fermented sample, ethanol, 2-methyl-3-pentanol and 4-methyl-1-pentanol increased during the first day of incubation. These alcohols could have originated from the activities of endogenous enzymes in meat and the residual enzymes from inherent microorganisms or garlic. Only 1-penten-3-ol decreased. 2-Butanol, 2-pentanol, 3-methyl-2-buten-1-ol and 3-methyl-3-buten-1-ol were absent in the non-fermented sample. These compounds, therefore, could have originated from microbial activity in the fermented samples. Other alcohols in the non-fermented sample remained unchanged. The total alcohol concentration in the non-fermented sample decreased after day 2.

The total concentration of alcohols in LC nham was constant after an increase on day 1. Alcohols in the natural fermented nham continued to increase until day 2 but decreased on day 3. Fermented nham samples showed a noticeable increase in ethanol, 1-propanol, 4-methyl-1-propanol and 1-hexanol. LC nham was lower in 2-butanol and 1-butanol when compared to the natural fermented sample but higher in 4-methyl-1-pentanol on the third day of fermentation.

Several alcohols in nham such as 2-propen-1-ol, 1-penten-3-ol, 1-propanol, 1-pentanol, 1-hexanol, 2-heptanol and 1-octen-3-ol were probably resulted from lipid oxidation. Ethanol and 2-butanol could have resulted from carbohydrate metabolism whereas the methyl- branched alcohols were generated from the breakdown of valine, isoleucine, and leucine. In addition, 1-butanol, 2 and 3-methyl-1-butanol could also be generated from the reduction of butanal, and 2 and 3-methyl-1-butanal, respectively (Frankel, 1991; Stahnke, 1994; Viallion *et al.*, 1996; Sunesen *et al.*, 2001).

Esters are important volatile compounds in fermented nham due to their low odour threshold values. The

concentrations of esters in the samples agreed with the numbers of LAB in Figure 2 indicating that microbial activity is crucial to ester formation in nham. In the fermented samples, esters increased considerably during the first day of fermentation and only slight change occurred thereafter. The following odour descriptions and odour thresholds of certain esters in water are from Flavor-Base version 2004 (Leffingwell and Associates, 2004). The most noticeably increased esters in nham were methyl butanoate (*sweet, ethereal fruity, threshold = 60-76 ppb*), ethyl acetate (*ethereal, sharp, wine-brandy like, threshold = 5 ppb*) and methyl hexanoate (*methyl caproate, ethereal fruity, pineapple-apple, threshold = 70 ppb*). The natural fermented nham had a higher level of total esters than the LC sample. This is probably because the indigenous microorganisms might have a higher esterase activity than *L. curvatus*. The LC nham contained much more ethyl butanoate (*ethereal, fruity, buttery and ripe fruit notes*) that has a very low threshold of 1 ppb, but it was lower in methyl butyrate than the natural fermented sample.

Esters in meat products are generally the result of esterification of carboxylic acids and alcohols (Shahidi *et al.*, 1986; Stahnke, 1994; Ansorena *et al.*, 2001). The esterification of acetaldehyde, propanal, butanal, pentanal and octanal leads to the formation of ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl hexanoate and ethyl octanoate, respectively (Stahnke, 1994). Low molecular weight esters, such as ethyl acetate, ethyl propionate, propyl acetate and ethyl butanoate, could be derived from carbohydrate catabolism (Kandler, 1983).

Stahnke (1994) showed that ethyl esters were associated with fruity aroma and that they mask rancid odours in fermented sausage. In our study, at the end of the fermentation the concentrations of ethyl caproate, ethyl acetate, ethyl 2-hydroxypropanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate in LC nham were higher than those in the natural fermented sample. This fact indicates that ethyl esters are important to the overall flavour of LC nham.

The amount of esters in the non-fermented sample was low and remained unchanged throughout the fermentation ($P > 0.05$).

The concentration of total ketones in the natural fermented nham sample was gradually reduced whereas that in LC nham was fairly constant. The main difference is that, in the natural fermented nham, 3-hydroxy-2-butanone (acetoin) steadily decreased throughout the fermentation. It seems possible that indigenous microorganisms can reduce ketones to alcohols at a faster rate than *L. curvatus*. In the non-fermented sample, ketones, especially 3-hydroxy-2-butanone, increased and later decreased. This indicates that 3-hydroxy-2-butanone was formed without microbial activity. Other ketones such as 3-methyl-2-butanone and 4-octanone, in both fermented nham samples were typical autoxidation products that increased during fermentation. The ketone 2-pentanone increased only in nham with *L. curvatus* but 2-butanone showed an increase in concentration in both fermented samples on day 1 but not in the non-fermented sample indicating that it was probably derived from lipolytic activity of microorganisms.

Aldehydes in all samples decreased during fermentation possibly due to their oxidation to alcohols. All samples were originally high in hexanal due to the oxidation that previously had occurred in the pork and pork rinds. Fermented as well as non-fermented nhams had a higher concentration of the typical autoxidation aldehydes, e.g. 2-butenal, pentanal, hexanal, heptanal, decanal, 2-octenal, octanal, *trans*-2-hexenal and 2-ethyl-*trans*-2-butenal

(Table 2). It should be noted that after the fermentation period, nham samples did not contain 3-methyl butanal and 2-methyl butanal that provides the aroma characteristics in dry fermented European sausages (Stahnke, 1995; Montel *et al.*, 1998). The absence of 3-methylbutanal in nham samples is probably due to the fact that 3-methylbutanal was reduced to 3-methyl-1-butanol and also probably because lactic acid bacteria have low proteolytic enzyme activity especially at a pH lower than 6 (Krockel, 1995; Montel *et al.*, 1995).

Microbial metabolism in nham could have reduced aldehydes to alcohols through alcohol dehydrogenase. Similar reaction in the non-fermented sample could occur to a lesser degree when catalysed by endogenous muscle enzymes and inherent microbial enzymes. The concentrations of aldehydes, therefore, were lower in the natural fermented sample and LC samples when compared to the non-fermented sample. 3–Methylbutanal and benzaldehyde could be derived through Strecker degradation from leucine and phenylglycine, respectively (Mottram and Edwards, 1983; Hinrichsen and Andersen, 1994). In LC nham, benzaldehyde decreased along the entire fermentation period.

Unlike in dry fermented sausage, only two volatile carboxylic acids, namely, acetic acid and butanoic acid were detected in nham. Acetic acid is formed by the catabolism of carbohydrates, lipids or amino acids (Frankel, 1982). In the non-fermented sample, the acetic acid originated from reactions catalysed by endogenous muscle enzymes. The highest level of acetic acid was present in the natural fermented nham. Nham with *L. curvatus* had the lowest pH indicating a high lactic acid concentration in the sample (Lücke, 1985). Butanoic acid, which Stahnke (1994) found contributed to a cheese odour in European fermented sausages, was detected only in LC nham. Since lactic acid is non-volatile, it could not be isolated by the DHS-GC-MS method used in this study.

The concentration of hydrocarbons in the natural fermented nham increased significantly ($P < 0.05$) during the first 2 days of fermentation but then diminished in the last day. While the concentrations of hydrocarbons steadily decreased throughout fermentation in LC nham. It is possible that enzymatic reactions from microorganisms in both fermented nham samples could have changed these hydrocarbons to methyl ketones. Thus, it seems that changes in volatile hydrocarbons are dependant on microbial activity since the amount of hydrocarbons in the non-fermented sample remained roughly unchanged during fermentation. Other hydrocarbons, such as xylene, toluene and 1,3,5–trimethylbenzene, could have been contaminants from the plastic wrapping materials (Stahnke, 1994) and animal feed (Berdagué *et al.*, 1993). However, despite the changes described during fermentation there was no significant difference in the amount of hydrocarbons among the three nham samples at the end of fermentation.

Odour active value (OAV)

Odour active values were determined by dividing the concentrations of volatile compounds with their threshold values. Selected aroma compounds that have their threshold values available in the literatures and have OAV > 1 are listed in Table 3.

From the available OAV data in Table 3, the most prominent aroma compound in nham samples was ethyl butanoate (*buttery, ripe fruit note*). Ethyl butanoate in LC nham on day 2 had the highest OAV of 9072. This correlated with the highest LAB count in LC sample on day 2 (Figure 2). The other potent aroma compounds in nham samples were dimethyldisulfide (*strong onion, cabbage-*

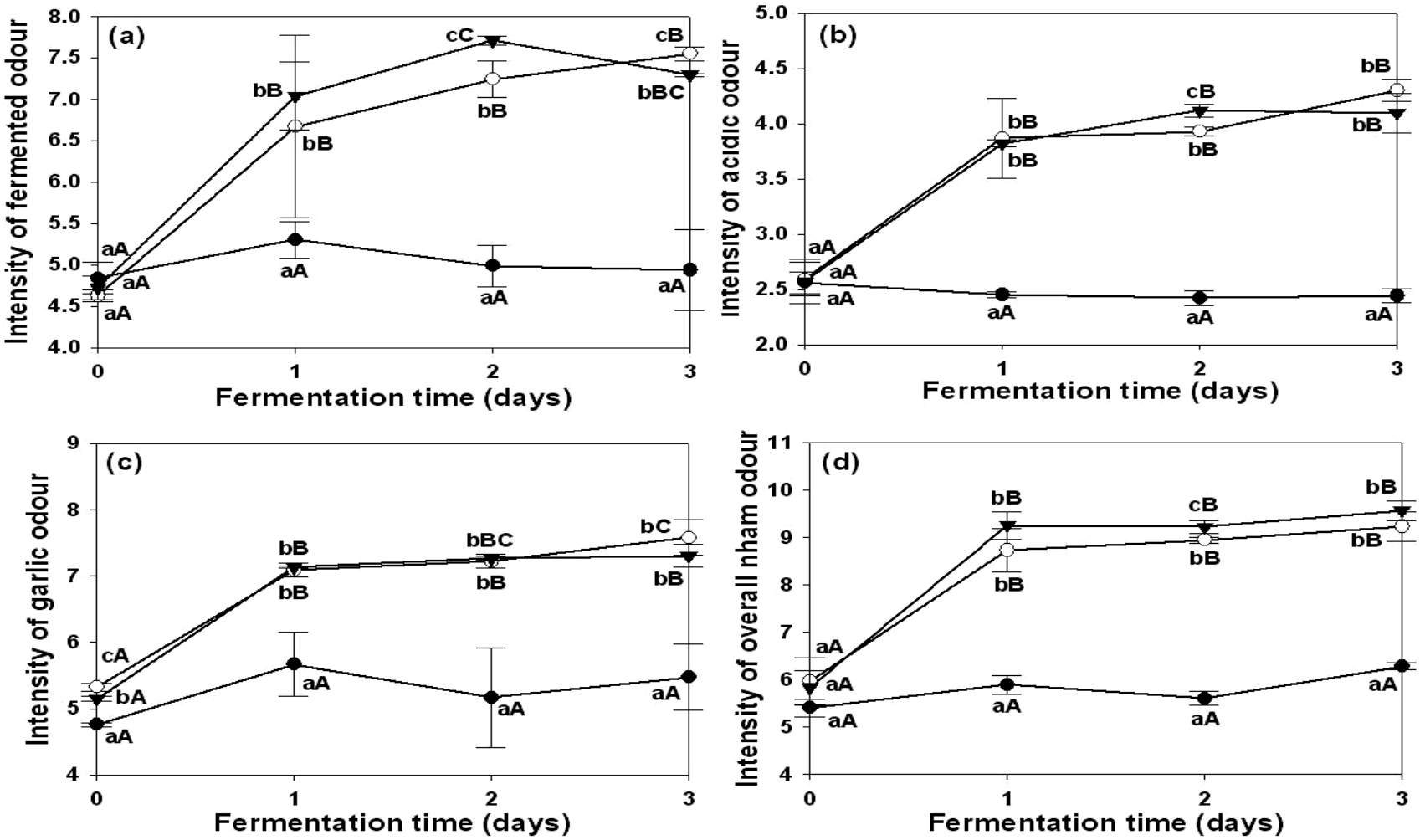

Figure 3. Changes in the intensities of (a) fermented, (b) acidic, (c) garlic and (d) overall nham odour during 0 to 3-day incubation of nham mixture with antibiotics (●), natural fermented nham (○) and nham fermented with *L. curvatus* (▼). a-c: Different letters in the same day are significantly different ($P < 0.05$). A-D: Different letters in the same sample are significantly different ($P < 0.05$)

Table 1. Average relative concentrations in headspace (ng/g) [a] of volatile compounds in natural fermented nham (N) and nham inoculated with *L. curvatus* (LC) during 3-day fermentation

Compound	RI [b]	R [c]	***Day 0***		***Day 1***		***Day 2***		***Day 3***	
			N	**LC**	**N**	**LC**	**N**	**LC**	**N**	**LC**
Hydrocarbons										
1,3-pentadiene	515	c	6.44	7.56	7.91	6.53	8.84	15.26	6.03	2.81
2-methylpentane	559	b	36.05	150.67	50.55	31.05	26.94	33.92	18.32	n.d.
3-methylpentane	576	c	13.73	112.70	19.29	20.54	20.60	12.51	9.70	3.39
methylcyclopentane	623	c	12.73	83.41	11.34	22.70	22.76	10.59	9.15	6.74
toluene	761	c	10.81	13.43	18.27	35.11	26.49	27.46	25.75	24.75
octane	800	b	5.74	5.72	22.45	24.92	23.15	20.89	23.63	18.94
p-xylene	875	b	n.d.	3.88	0.57	2.52	n.d.	2.26	0.86	1.71
1,2-dithiacyclopentane	986	c	6.56	3.80	8.94	6.44	3.87	3.47	2.86	2.17
1,3,5-trimethylbenzene	995	c	1.29	2.32	2.32	5.32	2.93	4.98	2.40	4.82
Subtotal			**93.35 aA** [d]	**383.49 bB**	**141.64 abB**	**155.13 bA**	**135.58 bB**	**131.34 bA**	**98.70 aAB**	**65.33 aA**
Aldehydes										
2-butenal	650	c	31.85	5.60	1.87	n.d.	n.d.	n.d.	n.d.	n.d.
3-methylbutanal	651	b	9.41	28.79	4.07	9.12	n.d.	n.d.	n.d.	n.d.
pentanal	717	c	4.60	13.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
hexanal	801	a	170.84	396.31	184.97	140.57	83.71	154.34	101.91	61.75
2-methyl-2-pentenal	826	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-ethyl-*trans*-2-butenal	856	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trans-2-hexenal	867	c	3.76	5.96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
heptanal	902	b	13.72	1.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
benzaldehyde	969	b	12.48	15.18	14.85	10.15	8.10	11.15	15.60	7.01
octanal	1001	b	12.62	13.40	17.65	19.13	11.87	20.02	12.15	12.49
2-octenal	1062	b	n.d.	10.94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
decanal	1204	b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Subtotal			**259.28** aA	**490.84** abB	**223.41** aA	**178.97** aA	**103.68** aA	**185.51** abA	**129.66** aA	**81.25** aA
Ketones										
2-butanone	603	b	83.89	83.75	428.91	363.37	301.39	221.63	164.49	190.97
3-methyl-2-butanone	658	c	19.83	14.13	23.19	20.70	24.95	18.82	21.37	24.84
2-pentanone	690	b	6.98	3.65	9.05	8.11	6.30	8.96	3.98	11.28

Compound	RI [b]	R [c]	*Day 0*		*Day 1*		*Day 2*		*Day 3*	
			N	**LC**	**N**	**LC**	**N**	**LC**	**N**	**LC**
3-hydroxy-2-butanone	720	a	820.10	720.26	121.91	469.62	79.86	448.31	22.36	424.84
2-heptanone	863	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-octanone	978	c	13.39	13.37	13.87	14.17	14.79	14.59	16.35	16.79
6-methyl-5-hepten-2-one	990	b	n.d.	4.98	3.61	2.72	2.35	1.27	n.d.	2.54
3-hydroxy-3-methyl-2-heptanone	1007	c	n.d.	9.94	15.99	12.44	n.d.	13.86	n.d.	n.d.
camphor	1156	b	12.58	7.13	20.85	9.33	5.04	11.78	6.41	2.68
Subtotal			**956.77 bB**	**857.21 bA**	**637.38 aAB**	**900.46 aA**	**434.68 aAB**	**739.22 aA**	**234.96 aA**	**673.94 bA**
Alcohols										
ethanol	<500	b	681.75	967.40	2460.65	2829.84	2835.67	2832.25	2750.41	2732.55
2-propanol	516	c	25.09	25.14	62.30	24.82	36.52	24.09	55.79	29.94
2-propen-1-ol	555	c	30.25	n.d.	13.15	n.d.	17.95	52.65	54.76	16.90
1-propanol	568	c	37.46	31.02	701.28	592.03	751.33	504.65	788.52	570.66
2-butanol	610	b	n.d.	n.d.	16.33	n.d.	872.71	n.d.	349.48	5.29
1-butanol	666	c	25.38	12.58	92.57	30.80	96.90	18.22	81.26	18.54
1-penten-3-ol	685	c	10.87	13.44	10.56	8.40	13.22	7.08	10.00	n.d.
2-pentanol	701	c	n.d.	n.d.	n.d.	n.d.	22.23	n.d.	15.56	3.68
3-methyl-1-butanol	736	b	13.36	16.95	10.51	80.30	35.60	77.38	28.18	89.08
2-methyl-1-butanol	738	c	n.d.	n.d.	n.d.	n.d.	n.d.	6.69	n.d.	4.75
3-methyl-3-buten-1-ol	742	c	6.53	n.d.	19.21	n.d.	18.51	n.d.	25.23	12.56
1-pentanol	769	b	35.77	57.45	38.52	33.08	21.20	22.82	18.39	25.42
3-methyl-2-buten-1-ol	779	b	n.d.	n.d.	n.d.	n.d.	7.85	n.d.	3.80	n.d.
2-methyl-3-pentanol	805	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-methyl-1-pentanol	847	c	n.d.	n.d.	178.35	98.40	114.84	114.85	138.08	345.39
1-hexanol	876	b	30.97	69.28	109.76	43.57	97.57	62.79	120.25	136.23
2-heptanol	906	c	n.d.	n.d.	4.77	n.d.	3.57	n.d.	2.63	n.d.
2-methyl-3-heptanol	970	c	15.13	16.52	14.97	14.90	14.63	12.83	11.21	n.d.
1-octen-3-ol	982	a	11.07	18.52	7.78	9.87	7.76	8.52	2.94	5.44
Subtotal			**923.63 aA**	**1228.30 aA**	**3740.71 aB**	**3766.01 aB**	**4968.06 cC**	**3744.82 bB**	**4456.49 bC**	**3996.43 bB**
Esters										
methyl acetate	528	c	124.18	80.32	150.74	184.56	46.57	167.98	109.02	30.89
ethyl acetate	616	a	n.d.	2.38	139.72	163.48	271.52	246.10	257.57	304.03
dimethyl carbonate	620	c	3.31	3.00	2.99	2.55	1.94	2.45	2.49	1.41

Compound	RI [b]	R [c]	***Day 0***		***Day 1***		***Day 2***		***Day 3***	
			N	**LC**	**N**	**LC**	**N**	**LC**	**N**	**LC**
methyl propanoate	630	c	11.76	12.96	10.88	19.10	10.19	9.08	10.46	14.93
methyl 2-methylpropanoate	681	c	4.73	n.d.	8.11	6.05	3.12	3.49	1.94	0.93
ethyl propanoate	714	a	n.d.	n.d.	n.d.	n.d.	3.49	8.95	3.24	n.d.
propyl acetate	716	b	n.d.	n.d.	10.48	n.d.	11.93	8.64	8.44	n.d.
methyl butyrate	722	b	14.59	25.36	625.04	296.01	722.30	124.73	374.76	59.13
ethyl 2-methylpropanoate	775	c	n.d.	n.d.	n.d.	n.d.	n.d.	3.09	2.52	3.74
methyl 3-methylbutyrate	776	c	n.d.	n.d.	34.79	n.d.	17.65	36.80	14.08	15.09
methyl 2- methylbutyrate	777	c	12.09	21.26	7.43	60.47	22.36	12.40	1.70	1.69
ethyl butanoate	804	a	n.d.	n.d.	20.02	124.36	19.35	294.00	44.29	168.04
ethyl 2-hydroxypropanoate	821	c	n.d.	n.d.	n.d.	n.d.	26.96	43.23	33.56	80.12
methyl valerate	830	c	6.01	6.62	13.87	15.17	12.43	11.09	8.87	6.80
ethyl 2-methylbutanoate	860	b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.69	5.31
methyl hexanoate	936	b	19.03	31.23	105.42	118.50	93.72	96.94	65.39	48.40
butyl 2-methylpropanoate	962	c	4.26	3.73	2.54	1.84	3.14	1.55	7.34	3.58
ethyl hexanoate	999	a	n.d.	n.d.	2.45	4.21	4.18	5.40	6.13	8.19
(E,E)-2-4-hexadien methyl ester	1017	c	3.17	n.d.	2.54	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-hexadienoic acid methyl ester	1019	c	n.d.	n.d.	n.d.	n.d.	n.d.	6.92	n.d.	n.d.
methyl heptanoate	1025	c	n.d.	n.d.	8.36	n.d.	9.34	7.78	n.d.	7.47
ethyl sorbate	1095	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
methyl octanoate	1119	c	2.33	4.18	4.79	3.38	4.98	2.14	4.27	3.69
ethyl octanoate	1155	a	n.d.	n.d.	n.d.	8.47	3.04	6.76	2.43	2.52
ethyl benzoate	1174	b	n.d.	n.d.	n.d.	n.d.	7.28	n.d.	8.81	n.d.
Subtotal			**205.46 bA**	**191.04 aA**	**1,150.17 cB**	**1,008.15 bB**	**1295.49 bB**	**1099.52 bB**	**971.00 bB**	**765.96 bB**
Sulfur containing compounds										
sulfur dioxide	<500	c	n.d.	11.21	6.13	5.33	5.92	4.69	6.34	3.33
2-propanethiol	550	c	0.84	2.23	4.05	3.37	4.85	3.96	4.36	4.11
2-propen-1-thiol	593	c	2,734.94	2,716.82	2,272.47	1,746.68	2738.40	3212.45	3776.98	4237.80
1-propanethiol	609	b	105.76	138.11	337.22	292.18	313.33	302.61	357.03	297.84
methylthiirane	637	c	31.30	36.13	410.81	505.14	650.84	669.52	763.61	800.64
thiophene	665	c	n.d.	n.d.	n.d.	1.30	n.d.	0.27	1.43	1.77
3-(methylthio)-1-propene	698	b	266.32	267.05	1675.41	1811.51	2244.31	2019.65	2146.92	2037.14
S-methyl ester-ethanethioic acid	702	c	108.73	68.56	145.40	98.92	53.07	53.66	34.30	45.12
1-(methylthio)-1-propane	712	b	3.12	n.d.	6.61	7.21	7.62	7.79	7.03	4.55
(*E*)-1-(methylthio)-1-propene	727	b	n.d.	n.d.	n.d.	n.d.	1.30	5.57	1.63	n.d.
(*Z*)-1-(methylthio)-1-propene	731	c	n.d.	n.d.	8.79	n.d.	9.01	19.56	6.22	10.37

Compound	RI [b]	R [c]	***Day 0***		***Day 1***		***Day 2***		***Day 3***	
			N	**LC**	**N**	**LC**	**N**	**LC**	**N**	**LC**
dimethyldisulfide	740	b	96.99	80.83	201.71	963.53	226.38	248.09	161.60	243.86
3-methylthiophene	767	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3,3′-thiobis-1-propene	866	c	4027.12	1217.69	4198.95	4544.13	5203.43	4967.87	5191.72	4761.33
S-propyl ester ethanetionic acid	877	c	n.d.	n.d.	53.94	27.21	47.84	17.72	36.68	11.91
3,4-dimethylthiophene	881	c	6.14	5.98	6.90	6.63	3.76	4.01	3.08	1.92
2,4-dimethylthiophene	903	c	21.00	25.03	14.58	14.78	12.96	14.64	12.47	9.08
methyl 2-propenyl disulfide	921	b	858.29	1266.06	2367.20	2410.03	2304.66	1719.33	1705.56	1686.84
methyl-trans-propenyl disulfide	937	c	1.85	2.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
methyl propyl disulfide	938	b	4.79	4.15	49.59	49.24	51.45	46.24	40.85	38.94
dimethyltrisulfide	974	b	19.75	34.44	21.22	26.02	12.20	14.20	4.93	9.75
di-2-propenyl disulfide	1088	b	4032.94	5266.63	7412.29	6989.01	6983.28	7361.66	6191.19	6034.35
2-propenyl propyl disulfide	1097	c	93.00	77.05	225.96	223.32	295.43	339.03	331.79	260.09
trans-propenyl propyl disulfide	1118	c	8.82	8.45	11.68	12.33	9.31	11.09	5.29	8.27
methyl 2-propenyl trisulfide	1148	c	355.09	484.30	396.37	417.11	275.37	336.51	152.49	221.13
3,4-dihydro-3-vinyl-1,2-dithiin	1202	c	22.76	20.57	51.55	36.66	52.29	45.59	39.12	45.00
2-vinyl-4*H*-1,3-dithiin	1230	c	37.75	40.49	65.54	55.35	50.83	57.04	38.23	33.68
2-vinyl-1,3-dithiane	1236	c	25.17	37.38	21.89	33.74	13.99	15.11	9.74	9.12
di-2-propenyltrisulfide	1322	c	291.98	305.32	476.53	397.96	334.84	380.26	268.88	244.42
Subtotal			**13,154.45** aA	**12,116.61** aA	**20,442.79** aAB	**20,748.69** aB	**21906.67** aB	**21878.12** aB	**21299.47** aAB	**21062.37** aB
Carboxylic acids										
acetic acid	638	c	6.16	6.90	21.36	17.90	30.14	21.42	36.66	30.53
butanoic acid	781	b	n.d.	n.d.	n.d.	5.02	n.d.	5.75	n.d.	5.98
Subtotal			**6.16**	**6.90**	**21.36**	**22.92**	**30.14**	**27.17**	**36.66**	**36.51**

Compound	RI [b]	R [c]	*Day 0*		*Day 1*		*Day 2*		*Day 3*	
			N	**LC**	**N**	**LC**	**N**	**LC**	**N**	**LC**
Terpene										
limonene	1031	b	21.31	20.38	15.17	28.49	25.60	21.82	13.28	13.41
Furan										
2-pentylfuran	993	b	5.48	4.70	5.74	6.10	8.41	6.51	9.55	8.71
Halogenated compounds										
dichloromethane	529	b	26.07	30.48	31.79	38.94	41.35	19.04	211.70	39.40
chloroform	617	b	46.01	88.05	29.62	28.37	9.87	7.58	8.18	n.d.
Subtotal			**72.08**	**118.53**	**61.41**	**67.31**	**51.22**	**26.62**	**219.88**	**39.40**
Miscellaneous compounds										
1,1′-oxybisethane	508	c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4,5-trimethyl-1,3-dioxolane	752	c	n.d.	n.d.	n.d.	n.d.	n.d.	10.09	n.d.	21.90
2-methyl-3,4-dihydro-2*H*-thiopyran	892	c	16.62	17.08	15.20	n.d.	n.d.	n.d.	n.d.	n.d.
1,8-cineole	1036	b	n.d.	n.d.	1.32	1.02	1.92	1.13	n.d.	0.74
Subtotal			**16.62**	**17.08**	**16.52**	**1.02**	**1.92**	**11.21**	n.d.	**22.64**

[a] Mean extracted quantity in ng 2-methyl-3-heptanone equivalents per g nham (n=2); n.d. = not detected.
[b] RI = retention index on the HP-5 capillary column.
[c] R = reliability of the identification. a, mass spectrum and RI identical with those of an authentic sample; b, mass spectrum and RI agreed with literature data; c, mass spectrum agreed with Wiley 275 library.
[d] a-c: Different letters within a row with the same day are significantly different ($P < 0.05$).
A-D: Different letters within a row with the same Nham sample are significantly different ($P < 0.05$).

Table 2. Average relative concentration in headspace (ng/g)[a] of volatile compounds in nham with antibiotics during 3-day fermentation

Compound	RI[a]	R[b]	Day 0	Day 1	Day 2	Day 3
Hydrocarbons						
2-methylpentane	559	b	n.d.	n.d.	5.70	27.48
p-xylene	875	b	n.d.	n.d.	6.58	7.50
1,2-dithiacyclopentane	986	c	21.41	5.55	3.93	2.44
1,3,5-trimethylbenzene	995	c	1.31	1.23	2.37	2.47
Aldehydes						
2-butenal	650	c	68.35	12.95	6.27	3.13
pentanal	717	c	14.84	n.d.	n.d.	n.d.
hexanal	801	a	471.12	207.61	224.51	130.65
2-methyl-2-pentenal	826	c	12.22	7.64	25.33	40.81
2-ethyl-*trans*-2-butenal	856	c	8.91	n.d.	n.d.	n.d.
trans-2-hexenal	867	c	5.52	n.d.	n.d.	n.d.
heptanal	902	b	12.04	n.d.	n.d.	n.d.
benzaldehyde	969	b	40.64	9.15	8.90	7.01
octanal	1001	b	26.78	22.19	n.d.	51.59
2-octenal	1062	b	17.21	n.d.	n.d.	n.d.
decanal	1204	b	1.96	n.d.	n.d.	n.d.
Ketones						
3-hydroxy-2-butanone	720	a	465.94	805.76	623.83	533.47
2-heptanone	863	c	n.d.	8.35	7.26	7.06
3-hydroxy-3-methyl-2-heptanone	1007	c	n.d.	10.20	11.11	11.96
Alcohols						
ethanol	<500	b	766.43	2,106.96	1,717.41	1,051.24
2-propanol	516	c	46.49	52.90	18.25	15.24
1-propanol	568	c	29.71	67.06	61.43	94.80
1-butanol	666	c	40.69	45.44	41.88	29.89
1-penten-3-ol	685	c	111.37	20.49	28.32	16.09
1-pentanol	769	b	56.98	48.99	12.02	n.d.
2-methyl-3-pentanol	805	c	n.d.	18.27	9.93	n.d.
4-methyl-1-pentanol	847	c	n.d.	93.58	162.28	171.07

Compound	RI[a]	R[b]	Day 0	Day 1	Day 2	Day 3
Esters						
methyl acetate	528	c	124.94	64.26	63.82	60.65
2,4-hexadienoic acid methyl ester	1019	c	63.17	69.33	97.53	59.56
ethyl sorbate	1095	c	n.d.	n.d.	28.53	24.90
Sulfur containing compounds						
sulfur dioxide	<500	c	7.09	6.16	5.70	13.49
2-propanethiol	550	c	n.d.	2.04	2.21	1.29
2-propen-1-thiol	593	c	2,873.46	2,532.03	2,051.66	1,777.57
1-propanethiol	609	b	135.23	139.52	319.31	87.87
methylthiirane	637	c	40.91	399.99	524.77	582.66
3-(methylthio)-1-propene	698	b	249.60	2,260.39	3,086.31	3,377.01
S-methyl ester-ethanethioic acid	702	c	44.11	11.66	n.d.	n.d.
1-(methylthio)-1-propane	712	b	3.68	3.67	4.16	2.06
(*Z*)-1-(methylthio)-1-propene	731	c	n.d.	n.d.	8.98	8.62
dimethyldisulfide	740	b	139.17	176.46	235.42	163.95
3-methylthiophene	767	c	n.d.	2.26	58.61	150.34
3,3′-thiobis-1-propene	866	c	1,276.93	4,082.05	6,266.69	6,917.89
3,4-dimethylthiophene	881	c	8.23	6.61	6.47	3.92
2,4-dimethylthiophene	903	c	24.39	16.57	16.70	14.25
methyl 2-propenyl disulfide	921	b	1,020.96	2,135.09	1,947.20	1,437.57
methyl-trans-propenyl disulfide	937	c	2.24	0.83	n.d.	n.d.
methyl propyl disulfide	938	b	2.32	48.78	66.55	51.53
dimethyltrisulfide	974	b	22.86	24.71	17.78	9.10
di-2-propenyl disulfide	1088	b	4,542.76	7,375.92	7,303.07	4,566.20
2-propenyl propyl disulfide	1097	c	56.75	340.96	234.97	231.16
trans-propenyl propyl disulfide	1118	c	7.31	17.03	18.57	7.59
methyl 2-propenyl trisulfide	1148	c	323.89	413.59	345.42	172.77
3,4-dihydro-3-vinyl-1,2-dithiin	1202	c	13.33	40.48	37.83	25.22
2-vinyl-4*H*-1,3-dithiin	1230	c	35.09	58.52	40.00	30.31
2-vinyl-1,3-dithiane	1236	c	58.12	25.37	20.82	15.07
di-2-propenyltrisulfide	1322	c	288.13	408.98	293.70	216.59
Carboxylic acids						
acetic acid	638	c	8.99	11.64	12.11	14.88

Compound	RI[a]	R[b]	Day 0	Day 1	Day 2	Day 3
Furan						
2-pentylfuran	993	b	n.d.	3.56	6.27	8.96
Halogenated compounds						
dichloromethane	529	b	21.22	63.89	81.51	149.61
chloroform	617	b	51.10	39.61	29.34	35.32
Miscellaneous compounds						
1,1′-oxybisethane	508	c	n.d.	20.22	n.d.	n.d.
2-methyl-3,4-dihydro-2*H*-thiopyran	892	c	16.14	25.67	18.87	n.d.
1,8-cineole	1036	b	n.d.	n.d.	2.14	n.d.

[a] Mean extracted quantity in ng 2-methyl-3-heptanone equivalents per g nham (n=2); n.d.= not detected.
[b] RI = retention index on the HP-5 capillary column.
[c] R = reliability of the identification. a, mass spectrum and RI identical with those of an authentic sample; b, mass spectrum and RI agreed with literature data; c, mass spectrum agreed with Wiley 275 library.

Table 3. OAV of selected volatile compounds (OAV>1) in non-fermented mixture (NF), nham (N) and nham inoculated with *L. curvatus* (LC) during 3-day fermentation

Compounds	Odor description [a]	Threshold (ppb)	OAV											
			Day 0			Day 1			Day 2			Day 3		
			NF	N	LC	NF	N	LC	NF	N	LC	NF	N	LC
Aldehydes														
3-methylbutanal	Powerful, penetrating; cheesey-sweaty-fruity in dilution	4 [b]	-	2	7	-	1	2	-	-	-	-	-	-
hexanal	Strong, penetrating, fatty-green, grassy unripe fruit odor	5 [c]	94	34	79	42	37	28	45	17	31	26	20	12

Compounds	Odor description [a]	Threshold (ppb)	OAV											
			Day 0			Day 1			Day 2			Day 3		
			NF	N	LC	NF	N	LC	NF	N	LC	NF	N	LC
heptanal	Fatty; in dilution sweet, fruity, nutty, fatty-cognac like	3 [c]	4	5	<1	-	-	-	-	-	-	-	-	-
octanal	Fatty-fruity odor	0.7 [c]	38	18	19	32	25	27	-	17	29	-	17	18
2-octenal	Peculiar fatty, green-grassy-leafy	0.2 [d]	86	-	55	-	-	-	-	-	-	-	-	-
Ketones														
3-hydroxy-2-butanone	Creamy-buttery, yogurt-like odor	14 [e]	33	59	51	58	9	34	45	6	32	26	2	30
Alcohols														
1-octen-3-ol	Very strong, sweet, earthy mushroom odor	1 [f]	-	11	19	-	8	10	-	8	9	-	3	5
Esters														
methyl butyrate	Sweet, ethereal fruity odor	9 [b]	-	<1	<1	-	8	4	-	9	1	-	5	<1
ethyl 2-methylpropanoate	Sweet, ethereal, fruity odor; apple note	0.1 [g]	-	-	-	-	-	-	-	-	30	-	20	30
methyl 3-methylbutyrate	Strong, fruity, ethereal, apple-like	4.4 [b]	-	-	-	-	8	-	-	4	8	-	3	3
methyl 2-methylbutyrate	Fruity, sweet, apple, berry, ripe and pineapple	0.93 [b]	-	13	23	-	8	65	-	24	13	-	2	2
ethyl butanoate	Ethereal, fruity odor; buttery, ripe fruit notes	0.18 [e]	-	-	-	-	617	3833	-	594	9072	-	1367	5183
ethyl 2-methylbutanoate	Strong, green, fruity, apple-strawberry odor	0.1 [g]	-	-	-	-	-	-	-	-	-	-	37	53
methyl hexanoate	Ethereal fruity (pineapple-apple)	39 [b]	-	<1	<1	-	3	3	-	2	2	-	2	1
ethyl hexanoate	Strong, fruity,	1 [g]	-	-	-	-	2	4	-	4	5	-	6	8

Compounds	Odor description [a]	Threshold (ppb)	OAV											
			Day 0			Day 1			Day 2			Day 3		
			NF	N	LC	NF	N	LC	NF	N	LC	NF	N	LC
	winey odor; apple, banana, pineapple notes													
Sulfur containing compounds														
1-propanethiol	Strong, sulfuraceous, onion, cabbage-like	3.1 [h]	44	34	45	45	109	94	103	101	98	28	115	96
dimethyldisulfide	Strong onion, cabbage-like	0.16 [i]	870	606	505	1103	1261	6022	1471	1415	1551	1025	1010	1524
dimethyltrisulfide	Sharp powerful onion-garlic like	0.008 [i]	2858	2469	4305	3089	2653	3253	2223	1525	1775	1138	616	1219
Furan														
2-pentylfuran	Ethereal rum; earthy beany with vegetable notes	6 [f]	-	<1	<1	-	<1	1	-	1	1	-	2	1

[a] Odor description from Leffingwell (2004).
Thresholds in water are from [b] Schnabel *et al.* (1988), [c] Teranishi *et al.* (1974), [d] Kossa *et al.* (1979), [e] Boonbumrung *et al.* (2001), [f] Buttery and Ling (1998), [g] Takeoka *et al.* (1989), [h] Aksoy (1983) and [i] Rychlik *et al.* (1998)

like) and dimethyltrisulfide (*onion-garlic-like*). Sulphur-containing compounds generally have low threshold values. It can be extrapolated that the other sulphur-containing compounds in Table 1 may also play an important role in nham aroma. The informations on the threshold values of specific sulphur-containing compounds were not available therefore the OAV of most sulphur compounds could not be obtained.

Other aroma impact compounds in nham were ethyl 2-methylbutanoate (*fruity*), ethyl 2-methylpropanoate (*fruity*), 3-hydroxy-2-butanone (*yoghurt-like*), octanal (*fatty-fruity*) and hexanal (*green*). These compounds together with ethyl butanoate could contributed to fermented aroma of nham.

Sensory analysis

The intensities of the flavour attributes, "fermented", "acidic", "garlic" and "overall flavour of nham" at different fermentation stages are shown in Figure 3. The aroma intensity of the non-fermented sample was the lowest and constant throughout the fermentation. There was a fermentation odour in the non-fermented sample. It could have been generated by the activities of endogenous meat enzymes or residual enzymes from microorganisms as well as from products of autoxidation.

The intensities of all flavour attributes of the fermented samples were remarkably developed during the first day of fermentation. The intensities were then roughly constant except for the fermented odour that continued to increase on day 2 for both fermented samples and continued to develop on day 3 for the natural fermented nham. On day 2 the intensity of fermented odour of LC nham was higher than that of the natural fermented sample and also the highest among the samples ($P > 0.05$). This can be associated with the concentrations and OAVs of ethyl butanoate in the samples.

It should be noted that, after one day of fermentation, the panelists perceived the higher garlic intensity in the fermented samples than the non-fermented sample even though sulphur compounds were found in comparable concentrations in all samples. The perceived intensity of garlic odour could be affected by the presence of lactic acid in the fermented samples. The acid produced by LAB caused damage to the garlic cells that allowed sulphur-containing compounds to migrate and volatilized into the headspace.

Conclusions

Most prominent aroma compounds in nham are ethyl butanoate and sulphur-containing compounds from garlic. Ethyl butanoate had the highest impact on nham aroma and was concomitant with the fermented aroma attribute. Ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, 3-hydroxy-2-butanone, octanal and hexanal were also contributed to the aroma of nham. Nham aroma developed on the first day of fermentation. The drop of pH to the acceptable level was slower, 2 days for LC Nham and 3 days for natural fermentation. Utilisation of *L. curvatus* starter culture can shorten fermentation time and also provided the highest ethyl butyrate resulting in the highest fermented aroma of LC nham on day 2.

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