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Changes in lipid composition and fatty acid profile of Nham, a Thai fermented pork sausage, during fermentation

Wonnop Visessanguan [a,*], Soottawat Benjakul [b], Siriporn Riebroy [b], Mongkol Yarchai [a], Wanaporn Tapingkae [a]

[a] National Center for Genetic Engineering and Biotechnology, 113 Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand
[b] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

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Abstract

Changes in lipid composition and fatty acid profile of Nham during fermentation were investigated. Total lipids of Nham were in the range 2–3%. The extracted lipid of initial Nham mix consisted mainly of triglycerides (TG), accounting for more than 75% of the total lipid, followed by phospholipids (PL) and a trace amount of diglycerides (DG) and free fatty acid (FFA). During fermentation, TG, DG and PL decreased with a concomitant increase in FFA, indicating lipolysis of Nham lipids during fermentation. Changes in fatty acids of the total lipids, non-polar and polar lipid fractions were observed during fermentation. In both total and non-polar lipid fractions, the major fatty acids found in a descending order were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids, which together accounted for 90% of the total fatty acids. Increases in fatty acid contents in both total and non-polar lipid fractions, were observed with a corresponding decrease in the quantity of fatty acids of phospholipids. As the fermentation proceeded, peroxide value generally increased while TBARS values decreased. Overall, lipid oxidation in Nham occurred during fermentation but did not cause the objectionable odour and taste in any Nham tested.

Keywords: Fermented pork sausage; Nham; Fatty acid; Lipolysis; Lipid oxidation

1. Introduction

Lipid is an important constituent, determining both functionality and sensory properties of processed meat products. Depending on the content, composition, and properties, lipids as well as their fatty acids, contribute to a wide range of quality attributes. The changes in lipid during processing, such as lipolysis and lipid oxidation, have a major impact, both desirable and deleterious for the final product quality of meat products. Lipolysis constitutes the prior step to free fatty acid autooxidation. Following the release of fatty acids, secondary reactions of fatty acids result in the development of numerous oxidation products, such as aldehydes, ketones and alcohols, that are responsible for the flavour characteristic of meat products (Berger, Macku, German, & Shibamoto, 1990; Bolzoni, Barbieri, & Virgili, 1996; Flores, Grimm, Toldrá, & Spanier, 1997; García et al., 1991; López et al., 1992).

Lipolysis and oxidation have been widely studied in dry sausage (Dainty & Blom, 1995) and dry-cured ham (Buscailhon, Gandemer, & Monin, 1994; Moltilva, Toldrá, Nadal, & Flores, 1994; Toldrá, Flores, & Sanz, 1997). Changes in the fatty acid composition in intramuscular fat during processing have been reported for "French" (Buscailhon et al., 1994), "Serrano" (Flores et al., 1997; Moltilva et al., 1994) and "Iberian" dry-cured hams (Cava et al., 1997; Ordóñez et al., 1996).

* Corresponding author. Tel.: +66 2 5646700x3250; fax: +66 2 5646707.
E-mail address: wonnop@biotec.or.th (W. Visessanguan).

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Lipid hydrolysis and oxidation can significantly differ, depending on the properties of raw ingredients and the manufacturing parameters, e.g. temperature, pH and time of processing of meat. However, changes in lipid and its oxidative stability during fermentation of Nham, a traditional fermented pork sausage of Thailand, have not been reported.

Nham is normally made of minced pork, shredded cooked pork rind, 2–3% NaCl, cooked rice, garlic and 100–125 ppm of sodium nitrite, mixed well and wrapped tightly in banana leaves or plastic bags. Fermentation of Nham generally takes 3–5 days at room termperature (~30 °C) without further ripening. Nham usually has a pH of 4.4–4.8 with titratable acidity values of 0.77–1.60% (Phithakpol, Varanyanond, Reunmaneepaitoon, & Wood, 1995). Fermentation of Nham remains indigenous, relying on adventitious microorganisms to initiate the fermentation. Valyasevi, Jungsiriwat, Smitinont, Praphailong, and Chowalitnitithum (2001) suggested that fermentation of Nham involved the successive growth of different microorganisms dominated by lactic acid bacteria (LAB). During the fermentation of Nham, lactobacilli (*Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus sake*) and pediococci (*Pediococcus acidilactici* and *Pediococcus pentosaceus*) have been shown to be the dominant microorganisms (Tanasupawat & Daengsubha, 1983; Tanasupawat et al., 1992; Valyasevi et al., 2001). LAB produce organic acids from carbohydrates and cause the pH drop, which contributes to Nham formation and the inhibition of undesirable microorganisms. *Micrococcus* and *Staphylococcus* are capable of reducing nitrate to nitrite, which is important in producing the characteristic pigmentation. Also, as a source of lipolytic and proteolytic enzymes, they may contribute to flavour formation. The objectives of this study were to monitor the changes in lipid composition and fatty acid profile of Nham during fermentation.

2. Materials and methods

2.1. Nham preparation

Lean meat obtained from a local retailer was trimmed of all visible fat and connective tissue. After trimming, the meat was minced though a 2-mm plate. Pork skin was trimmed of all visible fat and brought to boil in water to cook and ensure removal of pig hair from the follicle. After scalding, the de-fatted skin was finely shredded. Two batches of Nham were prepared. Minced pork (52%), shredded cooked pork rind (35%), sucrose (0.4%), garlic (4.3%), salt (1.9%), cooked rice (4.3%), sodium erythorbate (0.2%), trisodium polyphosphate (0.2%), monosodium glutamate (0.2%), whole bird chilli (2%), and potassium nitrite (0.01%) were thoroughly mixed. Samples of the pork sausage were extruded through the stuffing horn into a polyethylene casing with a diameter of 3.0 cm (approximately 200 g each) and sealed tightly. Samples were incubated for 84 h at 30 ± 1 °C and 50 ± 2% relative humidity. Samples were randomly sampled at every 12 h for microbiological and chemical analyses.

2.2. Microbiological analyses

Nham sample (25 g) was aseptically transferred to a sterile plastic bag and pummelled for 1 min in a stomacher (IUL Instrument, Spain), with 225 ml of 0.1% sterile peptone water. Appropriate decimal dilutions of the samples were prepared using the same diluent and 0.1 ml of each dilution was plated in triplicate on different growth media. The following media and incubation conditions were used: (1) nutrient agar (NA) incubated at 30 °C for 2 days for total viable count (TVC), (2) De Man Rogasa and Sharpe (MRS) agar incubated at 30 °C for 1–2 days for lactic acid bacteria (LAB) count, (3) mannitol salt agar (MSA) incubated at 30 °C for 2–3 days for staphylococci/micrococci count, (4) yeast malt agar (YM), pH 3.5, incubated at 25 °C for 3–4 days for yeast and mold counts.

2.3. Chemical analyses

In order to prepare the samples for analysis, after removing the outer casing, samples were thoroughly cut up and ground in a meat grinder (Osterizer, USA) until a homogeneous sample was obtained. Moisture, lipid, ash, and protein contents of the meat, rind, and Nham were determined according to AOAC (2000). Direct pH measurement was done using a standard pH meter (Mettler Teledo 320, Switzerland). The titratable acidity (TA) in samples was measured by the method of AOAC (2000) and expressed as % lactic acid based on dry weight.

2.4. Lipid extraction

The lipids were extracted from 25 g of ground samples with a solvent mixture of chloroform–methanol–distilled water (50:100:50, v/v) according to the method of Bligh and Dyer (1959). The extracted lipid was redissolved in chloroform and stored under nitrogen in the dark at −20 °C for further analyses.

2.5. Thin-layer chromatography/flame-ionization detection

The lipid composition of total lipid extract was determined using an Iatronscan MK-5 TLC/FID analyzer (Iatron Lab. Inc., Tokyo, Japan). Thin-layer chromatography (TLC) of the lipid extract was performed on silica-coated quartz rods (Chromarod-S III, Iatron Lab-

oratories, Inc., Japan), using a single step development system consisting of benzene, chloroform, and acetic acid (52:20:0.7, v/v). After development, the solvent was removed by flushing the rods with a stream of nitrogen and drying in an oven for 2–3 min before analyzing with the flame ionization detector. The sample analyses were carried out under the following conditions: flow rate of hydrogen, 160 ml/min; flow rate of air, 2000 ml/min; scan speed, 30 s/scan. Peak area was quantified and expressed as mg/g sample dry matter.

2.6. Fractionation of total lipids

The total lipid extract was separated into non-polar and polar lipid extracts by using Sep-Pak® silica cartridges (Water Corporation, Milford, Massachusettes, USA) as described by Juaneda and Roquelin (1985). The samples (50–70 mg of total lipid extract) were loaded on the top of the cartridges. Non-polar lipids were eluted with 50 ml of chloroform. Thereafter, the polar lipid fraction containing the phospholipids was eluted with 30 ml of methanol. The lipid composition of both fractions was analyzed by TLC–FID.

2.7. Free fatty acid content

Free fatty acid (FFA) content was determined by the method described by Lowry and Tinsley (1976). Extracted lipid samples (20–30 mg) were evaporated to dryness and redissolved in 5 ml isooctane, added with 1 ml of cupric acetate–pyridine reagent, and mixed vigorously for 90 s using a vortex mixer. The upper phase was collected and absorbance was read at 715 nm using a spectrophotometer (Cary model, Japan). FFA was calculated from the standard curve of palmitic acid and expressed as mg palmitic acid/g lipid.

2.8. Fatty acid composition

The fatty acid profile was determined by fatty acid methyl ester (FAME)/gas chromatography using acetyl chloride as a reagent for transesterification according to the method of Christie (1993). The lipid sample (5 mg) was treated with 1 ml methanol:benzene (3:2, v/v) and 1 ml acetyl chloride: methanol (1:20, v/v). Then 50 μl magaric acid (0.5 μg/μl), used as internal standard, were added. The mixture was heated in a waterbath at 100 °C for 1 h in a nitrogen atmosphere. After cooling to room temperature, 1 ml of hexane and 1 ml of distilled water were added to extract the fatty acid methyl ester. The hexane phase was collected into a vial. All FAMEs were separated and quantified using a gas chromatograph equipped with autosampler and on-flame ionization detector (Shimadzu Model GC-17A, Japan). Separations were accomplished on a 25 m BPx70i capillary column (SGE Australia, Ltd., Australia) with 0.25 mm i.d. and 0.25 μm phase thickness. Column oven temperature was programmed from 150 to 220 °C. The injector and detector were maintained at 250 and 260 °C, respectively. Carrier gas was helium and the make-up gas was nitrogen. Identification of fatty acids was achieved by comparing the relative retention times of FAME peaks from samples with standards from Supelco (Bellefonte, PA, USA).

2.9. Peroxide value

The formation of primary products of lipid oxidation (peroxides) was evaluated on an aliquot of the fat extract according to the method of Low and Ng (1987). Extracted lipid was dissolved in 10 ml chloroform–acetic acid mixture (2:3, v/v), treated with 1 ml of saturated potassium iodide (KI) solution, and kept in the dark for 5 min. The mixture was treated with 20 ml of distilled water and shaken. One ml of starch solution (1.5% w/v) was added as an indicator. The peroxide value (PV) was determined by titrating iodine liberated from potassium iodide with sodium thiosulphate solution. The PV was defined as the reactive oxygen content, expressed as millimoles of free iodine per kg of lipid.

2.10. Determination of thiobarbituric acid-reacive substances

Thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Nham sample (5 g) were homogenised with 25 ml of 0.0375% TBA, 15% TCA, and 0.25 N HCl stock solution. The mixture was heated in boiling water for 10 min, followed by cooling with running water. The mixture was centrifuged at 5500 rpm for 25 min using a centrifuge (Hitachi, Tokyo, Japan). The supernatant was collected and absorbance was read at 532 nm using a spectrophotometer (Cary model, Japan). TBARS was calculated from the standard curve of malondialdehyde and expressed as μg malondialdehyde (MDA)/g sample dry matter.

2.11. Statistical analyses

Data were subjected to analysis of variance (ANOVA). Mean comparison was carried out by Duncan's multiple range test (Steel & Torrie, 1980).

3. Results and discussion

3.1. Chemical composition

Chemical compositions of Nham, before and after fermentation, are shown in Table 1. Except for fat content, no marked differences in the composition of

Table 1
Proximate composition of Nham before and after fermentation

Sample	Composition (% by weight)			
	Moisture	Ash	Protein	Lipid
Nham at 0 h	70.90 ± 0.09a	2.83 ± 0.18a	20.3 ± 0.15a	2.33 ± 0.06a
Nham at 72 h	71.23 ± 0.24a	2.69 ± 0.08a	20.4 ± 0.49a	2.62 ± 0.01b

Mean values and standard deviations with different letters (a,b) in the same column indicate significant differences ($P < 0.05$).

Nham were observed at the end of fermentation ($P > 0.05$). As a major constituent, water accounted for approximately 71%, followed by protein (20%) and trace amounts of ash and lipid. Nham proteins mainly originated from minced pork and cooked pork rind, two major ingredients in Nham raw mix, whereas ash content mainly came from salt and others additives. Lipid of Nham was in the range 2–3%. Compared to those reported by Lodge, Sarkar, and Kramer (1978), the values obtained were in agreement with those found in pork meat (2.9–3.2%). Nevertheless, they were obviously lower than those of other fermented sausages, which were generally up to 50% (dry basis). Since lean meat and cooked pork rind, used in Nham, contained no visible fat, lipids were probably derived from intramuscular lipids from minced pork and cooked pork rind.

3.2. Microbiological changes and fermentation characteristics of Nham

Changes in dominant microorganisms during fermentation are shown in Fig. 1(a). Microbial loads of Nham raw mix were within the range 10^6–10^7 CFU g^{-1}. From the results, initial flora of the Nham was dominated by LAB, with lower counts of staphylococci/micrococci, yeasts, and molds. Similar to the results of Khieokhachee et al. (1997), initial flora of the Nham derived mainly from the raw materials. The number of LAB increased drastically to a maximum of 10^8–10^9 CFU g^{-1} within 24 h and remained constant until the fermentation was completed. Lactobacilli are the major producers of lactic acid, responsible for the decrease in pH and the increase in acidity during the fermentation (Valyasevi et al., 2001). Following the growth of LAB, TA

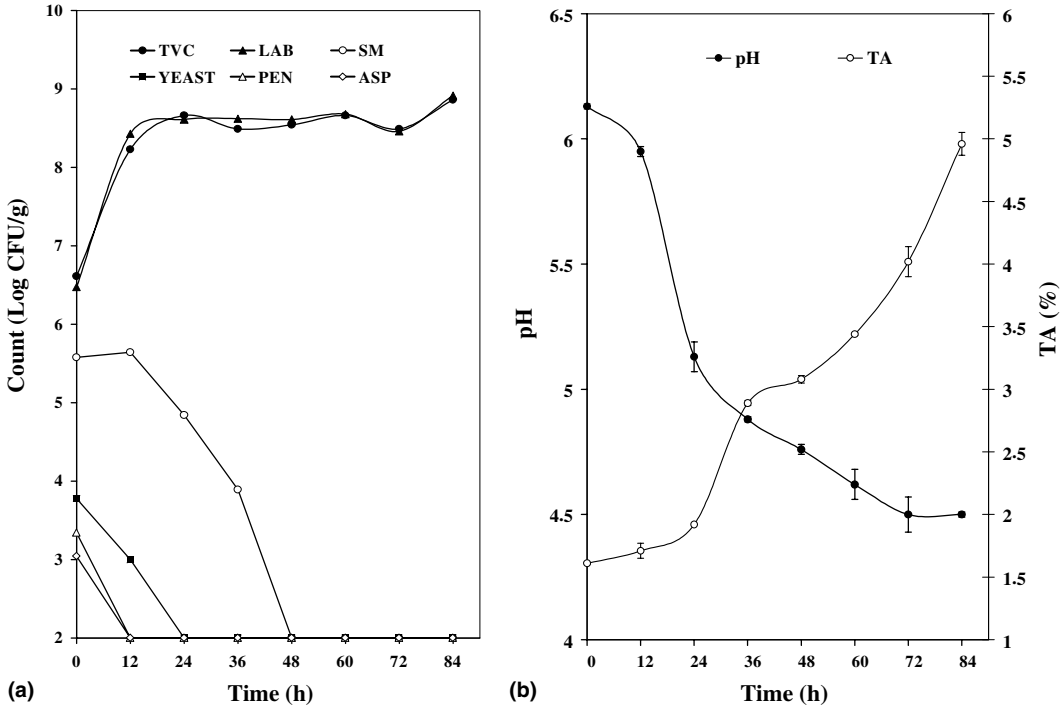

Fig. 1. Microbiological changes (a) and fermentation characteristics (b) of Nham during incubation at 30 °C. TA, titratable acidity; TVC, total viable counts; LAB, lactic acid bacteria; SM, Staphylococci/micrococci; YEAST, yeasts; PEN, Penicillium; ASP, Aspergillus.

continuously increased as fermentation time increased and reached a maximum at 72 h (Fig. 1(b)). With the increased TA, the pH of Nham gradually decreased to 4.6 within 72 h. Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the development of the Nham's texture of fermented sausage (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). The number of micrococci, with an initial level of 10^6 CFU g^{-1}, decreased to less than 10^2 CFU g^{-1}at 48 h, possibly due to decrease in pH and oxygen limitation. Yeast and mold also decreased rapidly and their counts were less than 10^2 CFU g^{-1} within 24 h. Micrococci/staphylococci and yeasts, in spite of their lower number compared to LAB, played a significant role in producing the characteristic pigmentation (Vernam & Sutherland, 1995) and the production of flavour compounds (Coretti, 1977).

3.3. Changes in lipid composition

Lipid of initial mix had triglycerides (TG) as a major constituent, accounting for more than 75% of the total lipid (Table 2), followed by phospholipids (PL) and a trace amount of diglycerides (DG) and free fatty acid (FFA). TG is located in fat cells along the muscle fibres and in small cytosolic droplets (Cassen & Cooper, 1971). Although the contribution of TG to the development of flavour remains low, TG is a good solvent for aroma compounds and plays a key role in aroma retention in meat products (Gandemer, 1999). With increasing time, TG, DG and PL decreased with a concomitant increase in FFA. The results suggested that the hydrolysis of TG and PL might take place, caused by both endogenous and microbial lipases or phospholipases. Separation of total lipid extract on Sep-Pak® silica cartridge confirmed a significant decrease in the contents of both non-polar and polar lipid fractions during the fermentation of Nham (data not shown). However, the lipolysis in Nham in fact was rather poor compared to other fermented sausages. Depending upon the type and processing of sausage, the degree of degradation is variable. Molly et al. (1997) showed that the degree of lipolysis in dry fermented sausage was higher in the triglyceride fraction than in the polar lipid fractions. Buscailhon et al. (1994) did not find any differences in the amounts of glycerides in dry cured ham, whereas they found a decrease in the amount of phospholipids. Navarro, Nadal, Izquierdo, and Flores (1997) reported a decrease in phospholipids in dry cured sausages ripened at 16 °C but no changes were detected when the drying temperature was 8 °C.

3.4. Free fatty acid content

FFA content at the beginning of the process represented 0.3% of the lipid content and increased to 3% at the end of fermentation (Fig. 2). Greater free fatty acid content was observed with increasing fermentation time, indicating lipolysis of Nham lipids during fermentation. Lipolytic activity at the beginning of fermentation was attributed to both lipases of the muscular tissue and microbial origin (Toldrá & Flores, 1998). Fermentation of Nham involved successive growth of different microorganisms dominated by lactic acid bacteria (LAB). However, Lactobacillus species are generally weakly lipolytic (Montel, Masson, & Talon, 1998). Selgas, Sanz, and Ordóñez (1988) showed the ability of the genus Micrococcus to hydrolyse triglycerides with long chain fatty acids, which are the most abundant in meat products. Since microbial lipases generally are very sensitive to pH, lipolysis at conditions relevant to Nham fermentation was likely mediated by lipases present in lysosomes of the muscle tissues. Molly, Demeyer, Civera, and Verplaetse (1996) confirmed the importance of endogenous lipase activity in Belgian sausages. These enzymes were still active even at the end of fermentation, and could be responsible for the decrease in phospholipids in this product.

3.5. Fatty acid composition of Nham

Fatty acid compositions of the total lipids, non-polar and polar lipid fractions of Nham were shown in Table 3. Similar fatty acid compositions were observed

Table 2
Changes in lipid composition of Nham during fermentation

Time (h)	Composition (mg/g dry matter)			
	TG	FFA	DG	PL
0	42.4 ± 0 38c	0.32 ± 0.03a	0.82 ± 0.04b	11.2 ± 0.39b
12	41.5 ± 0.07b	0.45 ± 0.02b	0.78 ± 0.02a	10.8 ± 0.06b
24	41.6 ± 0.27b	0.65 ± 0.03c	0.86 ± 0.07b	11.5 ± 0.36b
36	41.1 ± 0.08b	0.80 ± 0.00d	0.77 ± 0.00a	11.0 ± 0.08b
48	41.1 ± 0.15b	0.91 ± 0.02e	0.74 ± 0.00a	10.4 ± 0.13b
60	41.7 ± 0.48b	1.27 ± 0.04f	0.71 ± 0.04a	10.8 ± 0.56b
72	39.7 ± 0.54a	1.49 ± 0.01g	0.78 ± 0.01a	9.83 ± 0.56a
84	40.1 ± 0.17ab	1.66 ± 0.03h	0.74 ± 0.03a	9.39 ± 0.17a

Mean ± SD of two samples, each one in triplicate. Different letters (a,b,c,d) in the same column indicate significant differences ($P < 0.05$).

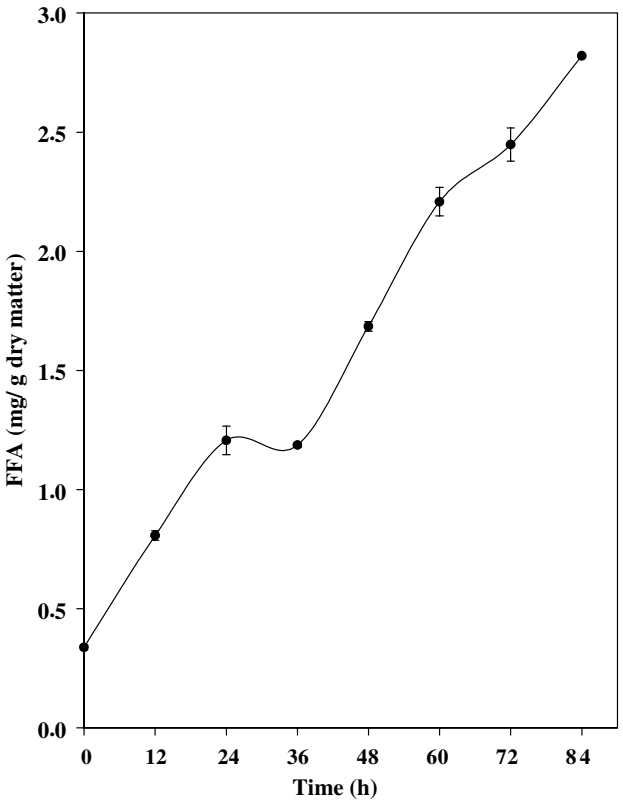

Fig. 2. Changes in free fatty acids during Nham fermentation. Bars represent standard deviation of two samples, each one in triplicate.

between total and non-polar lipid fractions except C22:0 and fatty acids with the longer chains. In both total and non-polar lipid fractions, the monounsaturated fatty acids (MUFA) were the most abundant, followed by the saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), respectively. Compared to subcutaneous fat, intramuscular fat generally had a higher percentage of MUFA and lower percent of PUFA (Vázquez et al., 1996). In total lipid, the major fatty acids found in a descending order were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) which together accounted for 90% of the total fatty acids. These fatty acid profiles are in agreement to those reported both in pig muscle (Leseigneur-Meynier & Gandemer, 1991) and in dry-cured ham (Buscailhon et al., 1994). However, it should be pointed out that the relative proportion of PUFA could be considered high. The fatty acid composition of the intramuscular fat is affected by several factors, of which diet generally seems to be one of the most important (Nürnberg, Wegner, & Ender, 1998). Remarkably high oleic acid percentage (56.4%) and low linoleic acid percentage (5.7%) were reported in the glyceride fraction of Iberian pig and possibly were responsible for the typical sensorial characteristics of Iberian ham. The most abundant fatty acid in polar lipid fraction (which accounted for 2.1% of the lipid content) was linoleic acid (C18:2) followed by C22:0 (Table 3). Polyunsaturated fatty acids accounted for a much

Table 3
Fatty acid profile of total lipid extract of Nham during fermentation

Fatty acid (mg/g dry matter)	Total lipid extract			Non-polar lipid fraction			Polar-lipid fraction		
	0 h	36 h	72 h	0 h	36 h	72 h	0 h	36 h	72 h
C14:0	0.18 ± 0.00a	0.20 ± 0.00b	0.23 ± 0.00c	0.18 ± 0.00b	0.16 ± 0.00a	0.19 ± 0.00b	ND	ND	ND
C16:0	2.81 ± 0.01a	3.11 ± 0.00b	3.79 ± 0.01c	2.95 ± 0.01b	2.54 ± 0.01a	3.21 ± 0.01c	0.38 ± 0.01c	0.31 ± 0.00b	0.29 ± 0.00a
C16:1	0.41 ± 0.01a	0.46 ± 0.00b	0.54 ± 0.02c	0.43 ± 0.01b	0.36 ± 0.01a	0.45 ± 0.01b	ND	ND	ND
C18:0	1.08 ± 0.00a	1.15 ± 0.00b	1.42 ± 0.00c	1.10 ± 0.00b	1.01 ± 0.00a	1.22 ± 0.00c	0.15 ± 0.00b	0.11 ± 0.00a	0.11 ± 0.00a
C18:1	6.41 ± 0.02a	6.96 ± 0.00b	8.30 ± 0.03c	6.57 ± 0.02b	5.59 ± 0.02a	6.84 ± 0.02c	0.21 ± 0.00b	0.16 ± 0.00a	0.16 ± 0.01a
C18:2	3.27 ± 0.02a	3.62 ± 0.00b	4.14 ± 0.02c	2.61 ± 0.02b	2.45 ± 0.02a	2.98 ± 0.02c	0.73 ± 0.02c	0.50 ± 0.00a	0.55 ± 0.00b
C20:0	ND	0.47 ± 0.00a	0.54 ± 0.00b	0.20 ± 0.00a	0.19 ± 0.00a	0.22 ± 0.00b	ND	ND	ND
C20:1	0.13 ± 0.00a	0.14 ± 0.00a	0.16 ± 0.00b	ND	ND	ND	ND	ND	ND
C20:2	ND	ND	ND	0.13 ± 0.00b	0.11 ± 0.00a	0.13 ± 0.00b	ND	ND	ND
C20:3	ND	ND	ND	0.19 ± 0.00b	0.14 ± 0.01a	0.22 ± 0.00c	0.05 ± 0.00b	0.02 ± 0.00a	0.04 ± 0.01b
C22:0	1.04 ± 0.01a	1.22 ± 0.00b	1.39 ± 0.00c	ND	ND	ND	0.68 ± 0.00c	0.29 ± 0.00a	0.66 ± 0.00b
C24:1	0.05 ± 0.00a	0.06 ± 0.00b	0.08 ± 0.00b	ND	ND	ND	0.03 ± 0.00a	0.02 ± 0.00a	0.03 ± 0.00a
C22:6	0.08 ± 0.01a	0.10 ± 0.00b	0.11 ± 0.00b	ND	ND	ND	0.11 ± 0.00a	ND	0.12 ± 0.00a
SFA	5.11	6.15	7.36	4.35	4.00	4.84	1.21	0.71	1.06
MUFA	7.00	7.62	9.08	7.00	5.95	7.30	0.24	0.18	0.19
PUFA	3.35	3.72	4 75	2.93	2.69	3.34	0.89	0.50	0.71
UNSAT	10.3	11.3	13.3	9.93	8.64	10.6	1.13	0.68	0.90
SFA/UNSAT	0.49	0.54	0.55	0.44	0.46	0.46	1.08	1.05	1.18

Mean ± SD of two samples, each one in triplicate. Fatty acids are represented in the following manner: the first number indicates the number of carbons, while the second represents the number of double bonds. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. ND: not detectable. Different letters (a,b,c,d) in the same row indicate significant differences within the same fraction ($P < 0.05$).

higher percentage (36–38%) than the former fraction (21%). The high levels of C22:0, C24:1 and C22:6, found in this polar lipid fraction, are noteworthy. Increased levels of C22:6 $n-3$ and C20:5 $n-3$ in pig muscle have been shown in several earlier studies, in which different amounts of fish oil were included in the diet (Irie & Sakimoto, 1992).

As the fermentation proceeded, increases of fatty acid contents, in both total and non-polar lipid fractions, were observed, with a corresponding decrease in the quantity of fatty acids of the polar lipid fraction (Table 3). Even though the effect on the composition of fatty acids was almost negligible, an increased amount of fatty acid in non-polar lipid fractions may contribute to the greater free fatty acid content (Fig. 2) and might partly come from the hydrolysis of phospholipids. Besides the lipolytic activity of both muscle and microbial lipases, the curing process generally resulted in an increase in saturated fatty acids (SFA), such as myristic, palmitic, and stearic acids, and decreases in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), e.g. oleic and linoleic acids (Martín, Córdoba, Ventanas, & Antequera, 1999). Igene, Pearson, and Gray (1981) and Buscailhon et al. (1994) found that most of the changes in lipid affect the polar fraction. Preferential specific release of PUFA from the polar lipid fraction was observed in dry fermented sausage (Molly et al., 1997). In Nham, a large increase in the amount of total UNSAT was observed. Due to their high susceptibility to chemical and enzymatic oxidation (Toldrá, 1998), unsaturated fatty acids possibly act as the precursors in flavour development in Nham during fermentation.

3.6. Lipid oxidation

Peroxide and TBARS values were used as indices to assess the level of lipid oxidation in Nham (Fig. 3). Relatively high peroxide and TBARS values at the beginning, indicated that oxidation of lipid occurred during processing. Shredding, mincing, and mixing of the meat, during the manufacture of the sausage, increased the surface area of the meat and exposed it to oxygen and oxidation catalysts (Gray, Gomaa, & Buckley, 1996). As the fermentation proceeded, peroxide value slightly increased while TBARS values decreased. Peroxide value measures the formation of peroxide or hydroperoxide groups that are initial products of lipid oxidation. Increase in peroxide value is marked when Lactobacillus is involved. Thiravattanamontri, Tanasupawat, Noonpakdee, and Valyasevi (1998) found that most of the lactic acid bacteria and other aerobic bacteria isolated from Nham

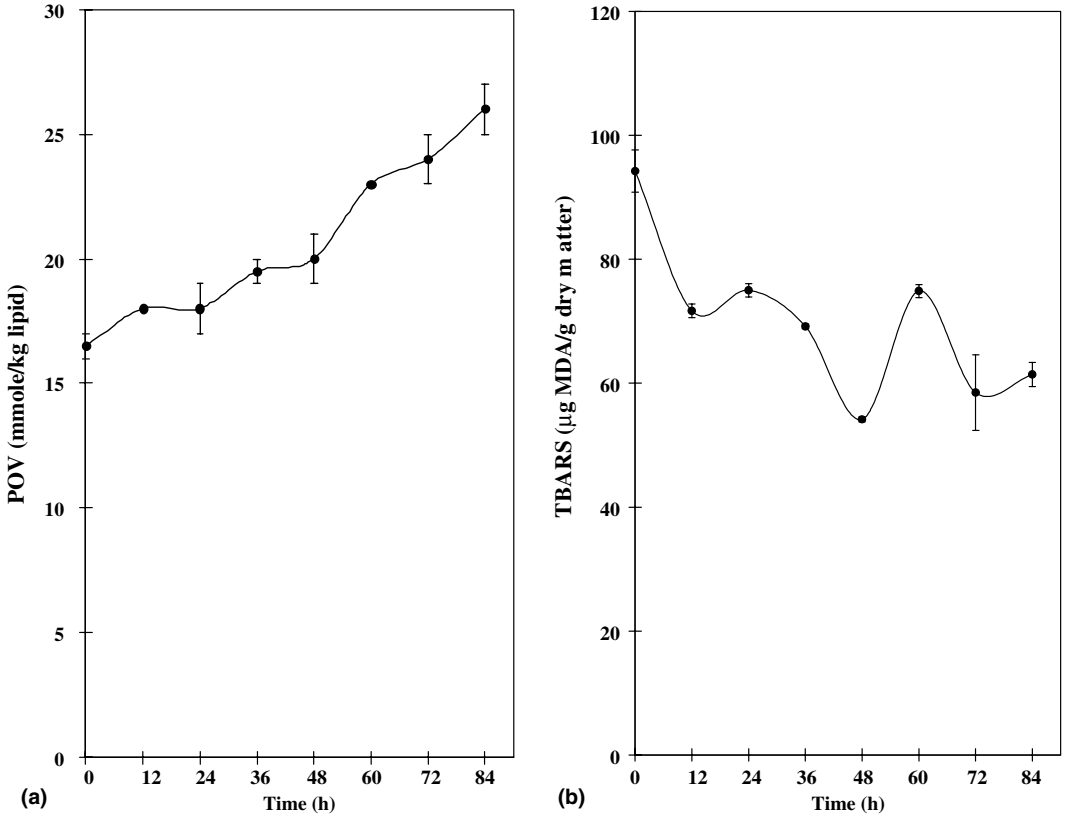

Fig. 3. Changes in peroxide (a) and TBARS (b) values of Nham during fermentation. Bars represent standard deviation of two samples, each one in triplicate.

produce hydrogen peroxide. Heme pigments, especially myoglobin, are responsible for meat colour and also are considered as strong prooxidants when they are activated by hydrogen peroxide (Harel & Kanner, 1985).

Overall, the oxidation level observed in Nham was relatively high when compared with those levels reported in good quality dry-fermented sausage and dry-salted ham (namely around 2–4 meq O_2 kg^{-1} fat for peroxide and 100–300 μg kg^{-1} tissue for TBARS). However, higher peroxide values (>20 meq O_2 kg^{-1} fat) were reported, during and at the end of maturing, in a superficial muscle in Iberian ham (Chizzolini, Novelli, & Zanardi, 1998). Nevertheless, no objectionable odour or tastes were reported in only Nham tested in this study (data not shown). This may be attributed to antioxidant activity of common ingredients used in Nham, including phosphate (Trout & Dale, 1990), nitrite (Freybler et al., 1993), and garlic (Yin & Cheng, 2003). Abnormally low TBA values are noticeable if some of the malonaldehyde reacts with proteins in an oxidizing system. In addition, the TBA method does not detect the oxidation of mono- or di-unsaturated fatty acids (Decker, Chan, & Faustman, 1998), while there are very large amounts of these fatty acids in beef, pork, mutton and poultry (Foegeding & Lanier, 1996). Gray (1978) reported that TBARS value correlated well with peroxide value only in oils containing fatty acids with three or more double bonds. No colour development was noted by the TBARS method for linoleate, a mono-unsaturated fatty acid, even though the peroxide value had already reached 2000.

4. Conclusion

The changes observed in lipid composition suggested lipolysis of both triacylglycerol and phospholipid during Nham fermentation. Overall, the amount of lipolysis was rather poor and the effect on the composition of fatty acids was almost negligible. The autooxidative phenomena, especially lipid oxidation, in Nham, was likely to be intense and might partially contribute to the Nham characteristics.

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