Lipid oxidation of Dutch style semi-dry fermented sausages prepared with beef and ostrich meat enriched with encapsulated linseed, algal and fish oils

Oxidación lipídica de embutidos fermentados semisecos estilo holandés preparados con carnes de res y avestruz enriquecidos con aceites encapsulados de linaza, algas y pescado

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Resumen— *Las salchichas fermentadas semi secas tipo holandesas fueron preparadas con aceites encapsulados de linaza, alga, y pescado, como sustitutos de la grasa dorsal del cerdo, y para aumentar los niveles de ácidos grasos poliinsaturados (especialmente los omega 3 o n-3). Las carnes de res y avestruz fueron utilizadas como ingrediente cárnico. La oxidación lipídica fue estudiada hasta los 60 días de almacenamiento del producto a 7 °C en una atmósfera modificada con un contenido de 63 % de oxígeno. El valor de peróxido (PV), las sustancias reactivas al ácido tiobarbitúrico (TBARS) y el hexanal fueron medidos como marcadores de oxidación y deterioro del producto. La evaluación sensorial fue llevada a cabo al final de los 60 días de almacenamiento por un panel entrenado de 11 miembros. El valor de peróxido disminuyó a los 60 días de almacenamiento bajo estas condiciones, debido a la inestabilidad de los peróxidos, generando productos secundarios de la oxidación, lo cual resultó en el incremento de las sustancias reactivas al ácido tiobarbitúrico y hexanal. La evaluación sensorial estuvo acorde con los valores obtenidos en los análisis de oxidación, encontrándose puntuaciones bajas en la aceptación de la mayoría de los productos después de los 60 días de almacenamiento, como resultado de la aparición de olor a pescado originado por la oxidación de los ácidos grasos n-3. La incorporación de carne de avestruz contribuyó a disminuir el contenido de grasa total y la adición de aceites con ácidos grasos n-3 incrementó el contenido de ácidos grasos poliinsaturados en el producto, haciendo disminuir a la vez, el contenido de ácidos grasos saturados. Sin embargo, este incremento en los ácidos grasos poliinsturados y el tipo de carne empleada en la elaboración de salchichas fermentadas tipo holandesas afectó negativamente la preservación del producto durante el almacenamiento debido a la susceptibilidad de estos a las reacciones de oxidación causando deterioro y pérdida de calidad del producto.*

Palabras claves— *Aceites encapsulados, ácidos grasos n-3, análisis sensorial, hexanal, oxidación lipídica, TBARS, valor de peróxido.*

Abstract— *Dutch style fermented semi-dry sausages were prepared using encapsulated linseed, algal and fish oil, as pork backfat replacers to increase the levels of Polyunsatured Fatty Acids (especially n-3). Beef and ostrich meat were used as the meat ingredients. Lipid oxidation was studied until 60 days of storage at 7 °C in a modified atmosphere containing 63 % of oxygen. Peroxide Value, Thiobarbituric Acid Reactive Substances and Hexanal were measured as markers of oxidation and deterioration of the product. Sensory evaluation was carried out at the end of 60 days of storage. PV decreased after 60 days of storage under these conditions, due to the instability of peroxides, generating secondary lipid oxidation products, which resulted in an increase of Thiobarbituric Acid Reactive Substances and Hexanal. Sensory evaluation was performed by a trained panel of 11 members, in accordance with values obtained in the lipid oxidation analyses, being low scores of acceptance in most of the products after 60 days of storage due to the presence of fishy smell originated from the oxidation of n-3 fatty acids. The incorporation of ostrich meat decreased to the total fat content and the addition of oils with n-3 fatty acids to these products increased PUFAs and decreased saturated fatty acid. However, this increase in PUFAs and the type of meat using in the elaboration of Dutch style semi-dry fermented sausages adversely affected the preservation of the product during storage due to the susceptibility of PUFAs to the lipid oxidation reactions causing deterioration and quality loss.*

Keywords— *Encapsulated oils, hexanal, n-3 fatty acids, lipid oxidation, peroxide value, TBARS, sensory analyses.*

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1. Introduction

The development of food that supports beneficial health effects including reduction of risks on diseases like cancer, cardiovascular diseases and obesity, is rapidly growing. A significant diet related risk factor for all these diseases is the amount and composition of fat intake. The present consumption of meat and meat products contributes substantially to the daily fat intake; however, sometimes it is not nutritionally optimal. Because of the composition of much animal fat, increased consumption of them has been associated with an increase in dietary ratio of n-6/n-3 polyunsaturated fatty acids and increased chronic disease [1].

As meat products are some of the most important sources of dietary fat, modification of the lipid profile of such products, by enhancing n-3 polyunsaturated fatty acids (PUFAs), by addition of oils such as linseed oil, fish oil, and other oils alter product fatty acid profiles producing "healthier" meat products, for example semi-dry fermented sausages [2], can help to improve the nutritional quality of the occidental diet [3]. To improve the health status of the population is recommended to regulate the consumption of food rich in n-3 PUFAs, in such a way that a n-6/n-3 PUFA ratio of less than 4 can be achieved and that the ratio of polyunsaturated and saturated fatty acids (P/S ratio) is higher than 0.4 [4].

The importance of PUFAs, especially the long chain n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is due to their biological function in human health and protective effect against some common cancers such as breast and colon cancer, rheumatoid arthritis and inflammatory bowel diseases [5], they are also implied in inflammatory processes and regulation of immune system [6]. Natural sources of long chain n- 3 PUFAs are fish oils, marine protists, dinoflagellates and microalgae [7].

Modification of the ingredients used for the elaboration of semi-dry fermented sausages has been tested previously [8]. However, total or partial replacement of animal fat in meat product by vegetable oils, or different sources, as encapsulated linseed oil, algal and fish oils, having increased PUFA contents, may contribute to a higher susceptibility to lipid oxidation, the primary process by which sensory quality declines in muscle foods [9]. The changes in lipids during processing and storage, such as lipolysis and lipid oxidation, have a major impact, both desirable and deleterious for the final product quality of meat products [10].

In semi-dry fermented sausages, being relatively high fat foodstuffs, lipid oxidation can negative influence their sensorial properties, by generation of degradation compounds such as n-alkenals and dienals, which are associated to rancid taste and odour. Oxidation can also affect the nutritional value of food by decomposition of vitamins, unsaturated essential fatty acids or can even give rise to toxic compounds [3].

The research as described in this paper is aimed to develop meat products based on a lower fat content and a better fatty acid profile by adding oils (encapsulated linseed, algal and fish oils) rich in essential fatty acid, especially n-3 fatty acids, and to study the effects of altered fat composition on lipid oxidation and sensory quality of the products.

2. Materials and methods

2.1 Sausage Preparation

Dutch style fermented sausages were manufactured at Wageningen University (The Netherlands). Lean beef, ostrich meat, pork backfat, encapsulated linseed, algal and fish oils were used as raw materials. The lean beef was from Ireland (Kepak Chuck Group, Ireland), the ostrich meat was from Namibia (Ostrich Production Namibia, LTD), and the encapsulated flaxseed oil (VANA-SANA ALA 30 ES), encapsulated algal oil (DHA KSF35) and encapsulated fish oil (VANA-SANA EPA/DHA 10/8 ES) were kindly provided by Kievit (Meppel, The Netherlands).

Table 1 shows the fatty acid profiles of the meats, pork back fat, and encapsulated oils, used in the different formulations.

Ten (10) formulations of fermented sausages of about 1.5 kg each were prepared. Four controls, traditional and low fat versions (two prepared with beef meat, sample codes C1 and C2 and two prepared with ostrich meat, sample codes S7 and S8, were produced using 30 % and 10 % pork backfat, respectively). C1 and S8 contain 30 % pork backfat and C2 and S7 contain 10 % pork backfat. Six formulations were produced with 10 % of added fat, in which pork backfat was replaced by 300 g encapsulated flaxseed, algal and fish oil, all containing 50 % of oil. Three samples were prepared with ostrich meat (S1, S2, and S3) and three samples were prepared with beef meat (S4, S5, and S6). The amount of the other ingredients in all formulations, expressed per 1.5 kg of meat mixture were: nitrite curing salt, 37.50 g; starter sausage (Lactobacillus curvatis), 15 g; glucose, 10.50 g; glutamate, 3 g; white pepper, 1.80 g; paprika powder, 1.50 g; crushed pepper, 1.50 g; ascorbic acid, 0.75 g; mace, 0.38 g; clove, 0.24 g; and garlic powder, 0.23 g.

The frozen meat (beef or ostrich) and pork backfat were cut, and the meat was chopped for 30 s in a FA-20 cutter (Stephan Nederland B.V., Almelo, The Netherlands) at low speed and 30 s at high speed and mixed with all other ingredients, except nitrite curing salt. The pork backfat was added and the meat mixture was chopped for 1 min at high

Table 1. Fatty acid profile of different meats and encapsulated oils (%)

Fatty acid	Meats			Encapsulated oils		
	Beef[1]	Ostrich[2]	Pork-back fat[3]	ALA 30 ES[4]	DHA KSF35[4]	EPA/DHA 10/8 ES[4]
C14:0	2.90	3.6	-----	-----	10.1	5.2
C16:0	23.90	22.2	22.1	5.0	23.2	12.4
C16:1	2.33	6.4	1.75	-----	2.1	4.7
C18:0	19.54	9.9	10.8	3.8	0.8	3.5
C18:1	35.30	34.3	39.1	18.9	2.8	6.0
C18:2	1.78	16.2	19.3	17.1	0.7	3.5
C18:3	0.63	2.4	1.34	54.1	-----	0.9
C20:1	-----	-----	0.88	-----	-----	1.6
C20:4n6	0.08	-----	-----	-----	0.6	1.4
C20:5n3	0.35	-----	-----	-----	1.5	23.8
C22:5n3	0.25	4.5	-----	-----	13.6	-----
C22:6n3	0.03	-----	-----	-----	35.6	18.4
Others	11.25	-----	-----	1.1	9.0	18.6
Σ SFA	53.21	36.8	34.9	8.8	34.1	21.1
Σ MUFA	41.99	40.7	42.8	18.9	4.9	12.3
Σ PUFA	4.81	23.1	22.3	71.2	52.0	48.0
P/S	0.09	0.6	0.63	8.1	1.5	2.3
n-6/*n*-3	1.55	2.3	12.63	0.3	0.4	0.1

Source: [1] Realini et al., 2005. [2] Broce, 2003. [3] Prieto et al., 2014. 4 Kievit (Meppel, The Netherlands).

speed. The obtained meat dough, encapsulated oils and nitrite curing salt were mixed in an N-506 Hobart mixer (Hobart MFG. Co., Troy, USA) at low speed for 1 min. After mixing, the meat dough was transferred into a plastic vacuum bag and air was removed with an Alvac-1-90 vacuum apparatus (Stephan Nederland B.V., Almelo, The Netherlands) and stuffed into 52 mm diameter cellulose based casings using a Dick, model TWF-12, stuffer (Friedr. DICK GmbH & Co. KG, Deizisau, Germany). The sausages were fermented for three days at 25 °C and dried at 15 °C and 65-80 % relative humidity for 12 days at Meester-Stegeman B.V. in Deventer (The Netherlands). To accelerate the rate of oxidation the sausages were cut in slices with a thickness of 6 mm and stored in the dark at 7 °C in modified atmosphere containing 63 % O_2, 22 % CO_2 and 15 % N_2. The packaging of the slices was carried out at PROMESSA in Deventer (The Netherlands), in polypropylene packages.

2.2 Chemical Analyses

2.2.1 Moisture

The moisture was determined in duplicate according to the official method for meat products [11].

2.2.2 Fat content

The total fat was quantified in duplicate according to the International Standard Meat and Meat Products [12].

2.2.3 Protein content

Protein was analyzed in duplicate with a NA 2100 Protein analyzer (CE Instruments, Milan, Italy) according to the Dumas Method described by Pelser et al. [8].

2.2.4 Fatty acid profiles

Fatty acids profiles were determined by gas chromatography. The fatty acid composition of the lipid fractions was determined in duplicate by preparing fatty acids methyl esters, as described by the International Organization for Standardization International Standards (1978).

Chromatographic conditions: GC-FID, HRGC 5300 (CE Instruments, Milan, Italy), equipped with a cold on-column injector and fitted with a capillary column 30 m x 0.53 mm ZB-wax; film thickness 0.50 µm (Phenomenex, Torrance, USA). The temperature of the detector was 270 °C, the oven temperature was programmed to increase from 80 to 160 °C with a rate of 15 °C min -1 followed by a rate of 7.5 °C min -1 from 160 to 240 °C and a final hold for 14 min. The carrier gas was helium at 60 kPa and the injection volume was 1 µl [8].

Standard used for identification was Supelco 47085-U, PUFA No. 3 (from Menhaden Oil), Sigma-Aldrich, Germany.

Fatty acid methyl esters were identified comparing retention time with standards and expressed as area % with respect to the total fatty acids.

2.2.5 Lipid Oxidation Analyses

2.2.5.1 Micro determination of Peroxide Values (PV)

Micro determination of Peroxide values was determined in duplicate as described by Asakawa and Matsushita [14] using a CARY 50 BIO UV-Visible spectrophotometer (Varian Analytical Instruments, Middelburg, The Netherlands) and reading at 560 nm.

2.2.6 Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances were determined in duplicate, as described by Alasnier et al. [15]. Analyses were carried out reading the samples at 535 nm in a CARY 50 BIO UV-Visible spectrophotometer (Varian Analytical Instruments, Middelburg, The Netherlands). TBARS were expressed as µg equivalent of malonaldehyde per kg of sample, using tetraethoxypropane as standard. A calibration curve was prepared.

2.2.7 Hexanal

The hexanal content was determined by a GC static headspace method according to Shahidi and Pegg [16]. 1.0 g of sample was transferred into 10 ml glass headspace vial. For the GC separation, an 8000 top GC (CE Instruments, Milan, Italy) with a capillary column Supelcowax (30 m x 0.54 mm; film thickness 1.0 µm, Supelco, Bellefonte, USA) and flame ionization detection (250 °C) was used. The oven temperature was raised linearly (7.5 °C min-1) from 60 to 80 °C and the final hold was 150 °C. Helium was used as a carrier gas with a pressure of 30 kPa and the injection volume was 1500 µl [8]. Analyses were done in duplicate.

2.3 Physical analyses

2.3.1 pH

pH measurements were carried out with a PHM82 Standard pH meter (Radiometer, Copenhagen, Denmark) on 10 g of sample homogenized with an Ultraturrax T 25 basic) (IKA-Werke, Staufen, Germany) with 10 ml of distilled water for 45 s.

2.3.2 Firmness

Firmness of the samples was measured using a texture analyzer (TA-XT2i/25, Stable Micro Systems, Etten-Leur, The Netherlands). Slices of sausages (6 mm thickness) were compressed in the central part to 50 % of their original height with a compression plate 30 mm in diameter at a speed of 5 mm/s. Compression force was taken as the maximum recorded force on the output expressed as Newton (N). The measurements were performed at room temperature [8]. For each formulation 4 determinations were carried out.

2.3.3 Color

Color measurements of the surface were obtained using a DRLANGE Tricolor LFM3 instrument (Dr Bruno Lange GmbH, Berlin, Germany). Results were expressed as L* (brightness), a* (redness) and b* (yellowness). For each formulation 4 determinations were carried out.

2.4 Sensory analyses

The sausages were sensory evaluated by an eleventh member trained sensory panel 60 days after production. Assessors were served with half a slice of sausage from each treatment with a thickness of 6 mm. The following attributes were tested: color intensity (redness), firmness (compression between thumb and index finger), and quality of texture (approximate texture in comparison to commercial sausages), smell intensity, quality of smell (approximate smell in comparison to commercial sausages), fishy smell, spicy smell and oiliness. A 5 point structured scale (1: minimum value, 5: maximum value) was used. The assessors also hedonically tested the sausages (11 sausages in total, including the commercial brand) and each one was ranged in the order of preferences (1: preferred, 11: not preferred).

3. Statistical analyses

A One-Way anova and Post Hoc test (Tukey) were performed to determine significant differences ($p < 0.05$) among the different types of sausages. The software used was SPPS version 15.0 (© 2006, SPPS inc., Chicago, Illinois).

4. Results and discussion

Table 2 shows the gross composition of the products (moisture, fat and protein content, and pH).

Control sausages C1 and S8 prepared with 30 % of pork backfat show the highest fat content in comparison to other formulations and these are consistent with the reported values from other authors for similar products [8, 17, 18]. The sausages in the present study prepared with 10 % fat can be considered as reduced fat according to the regulation (EC) N.º 1924 (2006) on nutrition and health claims made on foods (the reduced fat claim may only be made where the fat content reduction is at least 30 %) [19]. In the case of reduced fat fermented sausages, statistical differences ($p<0.05$) were found in moisture and fat content between sausages prepared with beef (S4, S5, S6) and ostrich meat (S1, S2, S3). However, sausages prepared with beef meat showed higher differences in total fat content in comparison to the sausages with ostrich meat. Differences were found in protein content between sausages and the pH values for these

products ranged 4.8 and 4.9. Mora-Gallego, Serra, Guàrdia and Arnau [19] found that the pH is significantly affected by the fat type level in reduced fat fuets, nonetheless, for Dutch style fermented sausages apparently this was not the case.

Table 2. Moisture, fat and protein contents (%) and pH values of different sausages

Samples	Physicochemical analyses			
	Moisture (%)	Fat (%)	Protein (%)	pH
C1	33.5^{a}	41.3^{g}	19.2^{a}	4.75^{a}
C2	42.2^{c}	22.7^{d}	24.8^{a,b,c}	4.78^{a,b}
S1	43.0^{c,d}	15.4^{b}	25.6^{b,c}	4.90^{d,e}
S2	38.3^{b}	10.4^{d,e}	23.5^{a,b}	4.95^{e}
S3	42.7^{c,d}	14.6^{b}	25.0^{b,c}	4.91^{d,e}
S4	38.7^{b}	24.9^{e}	21.2^{a,b}	4.85^{c,d}
S5	43.7^{d}	19.7^{a}	25.1^{b,c}	4.92^{d,e}
S6	38.0^{b}	23.9^{c}	22.0^{a,b}	4.87^{c,d}
S7	47.4^{e}	13.9^{b}	29.6^{c}	4.82^{b,c}
S8	37.8^{b}	33.6^{f}	21.6^{a,b}	4.84^{c,d}

See sausage preparation. ^{a,g} Means in the same column with different superscripts differ significantly ($p<0.05$).

The fatty acid profiles of the semi-dry fermented sausages are presented in table 3.

Table 3. Fatty acid profile of different dry fermented sausages

Fatty acid	Dry fermented sausages									
	C1	C2	S1	S2	S3	S4	S5	S6	S7	S8
C14:0	2.3^{c}	2.2^{c}	-----	5.9^{e}	6.6^{a}	1.4^{b}	5.9^{e}	4.3^{d}	1.3^{b}	1.5^{b}
C16:0	22.3^{c}	22.2^{c}	8.1^{b}	27.5^{f}	17.8^{e}	15.4^{d}	27.5^{f}	20.6^{a}	23.4^{g}	23.1^{g}
C16:1	2.6^{c}	2.3^{c}	-----	3.2^{d}	7.5^{a}	1.7^{b}	3.2^{d}	4.6^{e}	2.8^{c}	2.5^{c}
C18:0	12.4^{a}	12.2^{a}	5.2^{b}	12.3^{a}	6.0^{c}	11.4^{d}	12.0^{a}	19.2^{e}	12.4^{a}	12.1^{a}
C18:1	38.5^{c}	38.3^{c}	22.0^{d}	27.5^{e}	16.0^{b}	29.9^{f}	27.4^{e}	4.3^{a}	42.1^{a}	42.5^{a}
C18:2	11.1^{d}	11.6^{d}	17.6^{a}	26.4^{b}	4.7^{c}	10.9^{d}	2.7^{e}	25.6^{b}	16.0^{f}	15.6^{f}
C18:3	1.3^{b}	1.7^{b}	46.3^{a}	2.5^{c}	-----	28.6^{d}	1.4^{b}	2.9^{c}	1.4^{b}	1.3^{b}
C20:4n6	-----	-----	-----	1.3^{a}	2.2^{b}	-----	-----	2.1^{b}	1.1^{a}	0.6^{a}
C20:5n3	-----	-----	-----	-----	17.3^{b}	-----	-----	1.6^{a}	-----	-----
C22:5n3	-----	-----	-----	-----	2.5^{b}	-----	5.8^{c}	1.6^{a}	-----	-----
C22:6n3	-----	-----	-----	0.55^{b}	12.5^{a}	-----	14.2^{c}	10.6^{a}	-----	-----
Others	9.5^{a}	9.5^{a}	0.8^{b}	-----	6.9^{c}	0.7^{b}	-----	2.6^{d}	-----	0.8^{b}
Σ SFA	37.0^{c}	36.6^{c}	13.3^{b}	45.7^{a}	30.4^{c}	28.2^{d}	45.4^{a}	44.1^{a}	37.1^{c}	36.7^{c}
Σ MUFA	41.1^{b}	40.6^{b}	22.0^{c}	30.7^{d}	23.5^{c}	31.6^{d}	30.6^{d}	8.9^{e}	44.9^{a}	45.0^{a}
Σ PUFA	12.4^{b}	13.3^{b}	63.9^{a}	23.6^{d}	39.2^{e}	39.5^{e}	24.1^{d}	44.4^{f}	18.5^{c}	17.5^{c}
P/S	0.3^{b}	0.4^{b,c}	4.8^{a}	0.5^{c}	1.3^{e}	1.4^{e}	0.5^{c}	1.0^{d}	0.5^{c}	0.5^{c}
n-6/*n*-3	8.5^{d}	6.8^{c}	0.4^{b}	1.2^{e}	0.2^{b}	0.4^{b}	0.1^{b}	1.7^{c}	12.2^{a}	12.5^{a}

$^{a-e}$ Means in the same row with different superscripts differ significantly ($p<0.05$).

Differences were found in the control samples prepared with beef and ostrich meat (C1, C2, S7 and S8). All these sausages presented the highest content of C18:1 due to the presence of pork backfat in their formulations, since pork fat is rich in this specific fatty acid. Sausages prepared with ostrich meat presented higher values in C18:2 than those prepared with beef. Sausages S1 and S4 prepared with encapsulated linseed oil showed the highest content of linolenic acid. A main contribution of long chain fatty acids to the products is given by using fish oil and algal oil in the formulation. Sausages S2, S3, S5 and S6 present high amounts of DHA in comparison with the controls and linseed oil sausages. EPA was only found in the sausages S3 and S6 prepared with fish oil. Concerning to saturated fatty acids (SFA), highest contents were found in S2, S5 and S6 probably to the contribution of fat in meat and encapsulated oils used in the formulation of these sausages (see table 1). Enrichment of model products with encapsulated linseed, algal and fish oil increased considerably the amount of polyunsaturated fatty acids (PUFA) in all of the low fat fermented sausages. According to Valencia et al. [20], the partial or total replacement of pork backfat resulted in improved nutritional properties with regard to conventional sausages by an increased PUFA/MUFA ratio. Differences in ratio P/S were found in the samples, but all the sausages, including the controls, are within the recommended range [4]. The modified products showed better n-6/n-3 ratios than the control products from a nutritional point of view, satisfying the current recommendation for this ratio (< 4).

Table 4 shows the results in firmness and color (CIE L* a* b* system) of sausages at the beginning and after 60 days of storage. The modified sausages showed highest values in comparison to both controls with 30 % of pork back fat. This fact could be due to the increase in the amount of protein used during the preparation as a strategy to decrease the total fat content of the final product to 10 %. During the period of storage, no significantly differences ($p>0.05$) in firmness were observed in the formulations.

The controls, prepared with 30 % pork backfat, presented the lowest values of instrumental firmness.

Most of the sausages showed slightly lower values for Lightness (L*) than the controls prepared with beef and pork backfat at the beginning of measurements, with

exception of S3, which contained encapsulated fish oil, but these differences were not statistically significant. Valencia et al. [20] also found slightly lower values for lightness in fermented sausages. No significant differences were found in the sausages in the L* value during storage. C1 results in the highest L* value in comparison to the other formulations, probably due the content of pork backfat that contributes to a white color to the final product. In most of the cases redness (a*) decreased with storage time. This result can be a signal of color deterioration and lipid oxidation of the products. Values found for yellowness (b*) were consistent with Valencia et al. [20]. The higher values of b* results in a lower perceived color intensity, and higher values in fish oil containing sausages led to a more saturated red color (higher Chroma). No significant differences were found in the sausages during storage.

Table 4. Firmness and color (CIE L* a* b* system) of sausages during storage

Samples	Firmness (N*)		Color					
			Lightness (L*)		Redness (a*)		Yellowness (b*)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
C1	72.8	92.8	44.6	53.4	4.7	1.5	10.7	13.5
C2	111.3	56.0	44.1	43.3	3.2	0.6	6.7	6.7
S1	137.8	65.3	39.5	46.0	6.3	5.6	9.8	20.0
S2	153.6	86.9	40.5	42.9	10.2	4.1	11.1	11.5
S3	117.6	103.6	47.2	43.7	4.9	10.1	16.7	20.1
S4	119.2	149.2	43.5	51.1	6.8	2.8	9.3	15.2
S5	123.8	134.8	37.9	40.5	5.1	3.9	10.4	12.2
S6	122.5	106.7	39.3	43.3	9.1	9.7	8.5	20.5
S7	129.8	84.3	39.3	38.8	3.3	1.4	7.6	7.5
S8	48.2	49.6	40.7	45.7	2.8	2.9	10.6	16.6

Figure 1 shows the peroxide values measured (meq active O_2 kgv^{-1} sausage) of the different semi-dry fermented sausages at the start and after 60 days of storage at 7 °C in the dark and in 63 % O_2 atmosphere. Differences were found in all of the sausages at the beginning of the period of storage, highest values of peroxides (8.3 and 8.2 meq active O_2 kg^{-1} sausage) were found in the controls C1, C2 (30 % and 10 % pork back fat respectively), followed by control S8 (30 % pork back fat), showing a value of 5.1 meq active O_2 kg^{-1} sausage and S4 (10 % linseed oil), showing a value 3.7 meq active O_2 kg^{-1} sausage. These sausages presented a higher degree of primary oxidation in comparison to the other samples. This fact could be due to autooxidation reactions occurring in the raw materials and the presences of prooxidant substances in the food system, which cause or accelerate lipid oxidation [21]. Beef meat is rich in myoglobin that as well is a photosensitizer and can absorb energy from light to form an excited singlet state of oxygen. Singlet oxygen is considered as a prooxidant that promotes the formation of lipid hydroperoxides by matching the spin direction of the electron in the double bond, reacting with an unsaturated fatty acid directly to form lipid hydroperoxides. The samples containing mainly pork backfat in their formulation (C1, C2, and S8) showed the highest values, in comparison to the modified ones (possibly the encapsulated oil forms were protected by the presence of antioxidants, but this fact was not reported in the information data supplied by the company). After storage of 60 days the PV's decreased in most of the cases except in S2 and S5 (in this cases a slight increase in PV was shown).

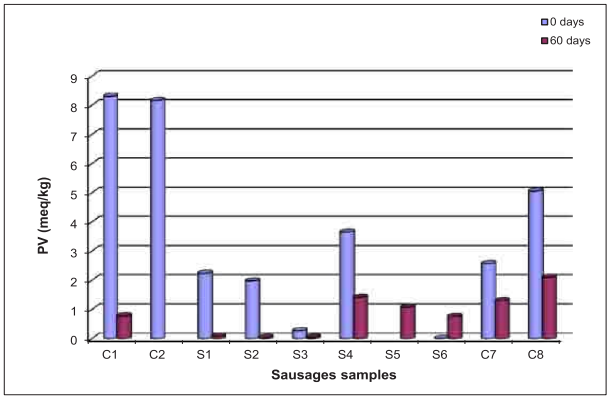

Figure 1. Peroxide value (meq active O_2/kg^{-1} sausage) of different dry fermented sausages during storage at 7 °C in the dark.

TBARS values (µg of malonaldehyde per kg of sausage), determining secondary lipid oxidation products, are presented in figure 2.

Figure 2 (section a) shows the results of TBARS obtained from sausages prepared with beef meat, pork back fat (control sausages C1 and C2, 30 and 10 % pork back fat respectively), and encapsulated oils (S4, S5, S6). An increase in TBARS numbers can be observed in all the sausages after storage of 60 days. At the beginning of storage period most of the sausages have lower TBARS values except C1 (control 30 % pork back fat) that showed the highest value in comparison to the modified ones and control S8 containing 30 % of pork back fat.

Nevertheless, these values are lower than 1 ppm (1000 µg kg $^{-1}$), which is considered to be the limit to detect rancidity [22]. At the end of the storage period sausages S4 and S6 showed the highest values. This fact could be to cause by the presence of polyunsaturated fatty acids in the formulation. Especially S6 (prepared with encapsulated fish oil) showed the highest TBARS due to its contents of C18:2, C18:3, C20:4n6, C20:5n3, C22:5n3, C22:6n3. The fact that the processes of mixing and stuffing are not

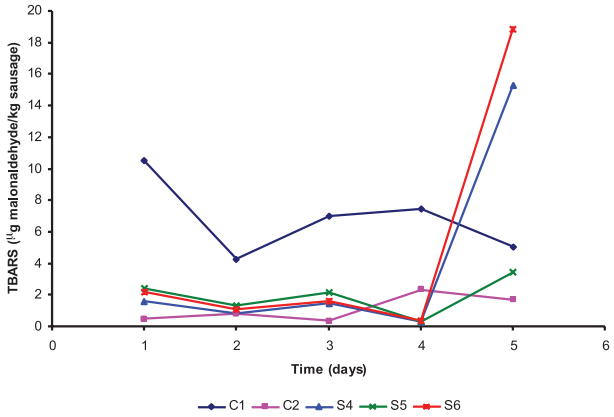

Figure 2a. TBARS values of different fermented sausages elaborated with beef meat during storage at 7 °C in the dark.

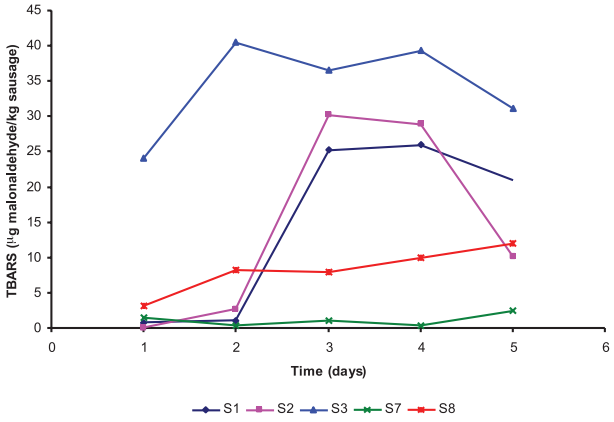

Figure 2b. TBARS values of different fermented sausages elaborated with ostrich meat during storage at 7 °C in the dark.

carried out under vacuum conditions, are decisive factors for the presence of oxygen in high concentration inside the sausage, which favors the development of autooxidation reactions generating a wide range of secondary oxidation products, as unsaturated aldehydes or malonaldehyde [22, 23]. An increase of TBARS during storage was also observed in the group of sausages prepared with ostrich meat and different fats. At the beginning sausage S3 showed the highest value of TBARS in comparison to the other formulations. This behavior could be due to contribution of ostrich meat and encapsulated fish oil, rich in n-3 fatty acids and susceptible to lipid oxidation, especially C20:5n3 and C22:6n3. Sausages S1 and S3 show the highest TBARS value at 30 days of storage and a decrease at 60 days. Lee et al. [25] reported for ground turkey patties and fresh pork sausages enriched with n-3, that TBARS values increase significantly with storage when no antioxidants are present in the formulations. One of the reasons could be the presence of salts in these products, which plays a role as prooxidant accelerating oxidation in sausages. Samples S7 and S8 showed the lowest TBARS values. In general it could be observed that the sausages manufactured with ostrich meat showed higher TBARS values than those prepared with beef. This fact could be due to the more unsaturated fatty acid profile of ostrich meat.

Hexanal values of the semi-dry fermented sausages are shown in figure 3.

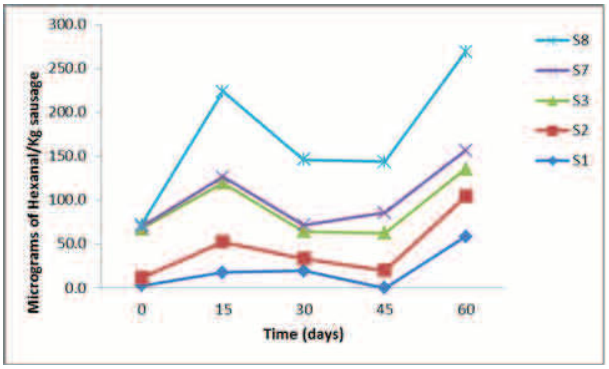

Figure 3a. Hexanal values of different fermented sausages elaborated with ostrich meat during storage at 7 °C in the dark.

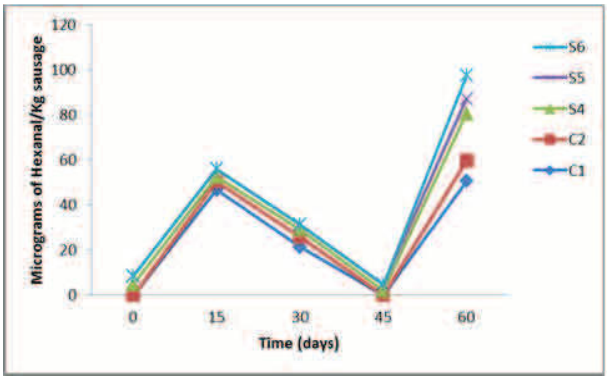

Figure 3b. Hexanal values of different fermented sausages elaborated with beef meat during storage at 7 °C in the dark.

Hexanal is often used as a marker for lipid oxidation in meats as a result of C18:2 n-6 oxidation [26]. At the beginning of the storage period most of the sausages prepared with ostrich meat, low hexanal values were found, with exception of S3 which showed the highest value at the starting point and decreased during storage. Sausages S8 and S1 underwent a similar behavior during storage, increasing their hexanal values reaching a maximum. According to Valencia et al. [23], hexanal increased during storage when emulsions with linseed oil were used to produce dry-cured meat sausages. The increase was dependent on the efficiency in excluding air during the process of manufacture and later in storage. Sausage S7 showed an increase of hexanal during storage reaching its maximum value at 45 days after, and then slightly decreasing at 60 days. In S2 hexanal was only found after 60 days of storage at the lowest value of the entire group, in spite of its fatty acid profile which contained more C18:2 (n-6), than the rest of the sausages.

According to the fatty acid profile S6 showed a high value in linoleic acid, which explains the presence of hexanal, due it is the principal precursor. If both groups are compared (ostrich meat and beef meat), sausages prepared with ostrich meat showed higher hexanal values than those prepared with beef meat. This could be due to the contribution of linoleic acid from ostrich meat and also due to the fact that poultry species appeared as the second most important factor, influencing the oxidative stability of the sausages due to their the fatty acid profile rich in polyunsaturated fatty acids in comparison to other animal species [28].

The results of the sensory evaluation after 60 days of storage are shown in the spider web diagrams of figure 4.

No differences were found by the panelists in relation to firmness in comparison to a commercial brand sample (CB) used as a control. On the other hand, controls C1 and C2 prepared with 30 % and 10 % pork backfat maintained relation with instrumental results for firmness. Both controls presented the lowest values in the end of the storage period at 60 days. In case of the texture quality, only S4 was evaluated by the panelist as moderately comparable as the commercial brand used as a reference sausage, the rest of the modified sausages were scored with lowest values in comparison to the reference. As reported by other authors, an increase in the content of PUFA may lead to "soft" meat and meat products of inferior quality [29]. The storage of meat and meat products reduced shelf life when

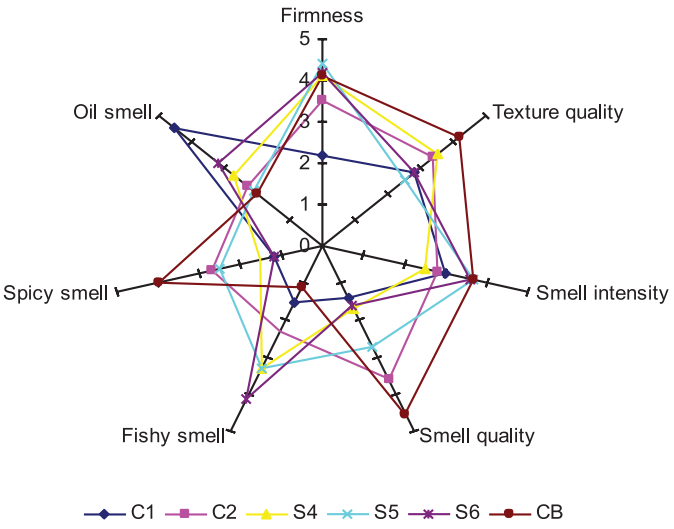

Figure 4a. Sensory evaluation of Dutch style fermented sausages manufactured with beef (scales from 1 to 5).

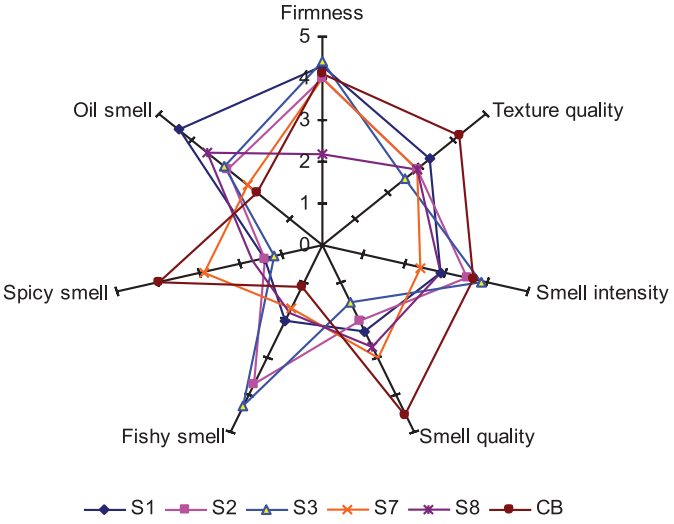

Figure 4b. Sensory evaluation of Dutch style fermented sausages manufactured with ostrich meat (1: minimum value, 5: maximum value).

unsaturated fatty acids are present in large amounts due to their high susceptibility to oxidation [30, 31, 32]. In case of smell intensity, assessors scored the samples S5 and S6 higher in comparison to the other samples after 60 days of storage. This observation is in agreement with the TBARS and Hexanal values. At this point the sensory panel could detect certain deterioration of the products, especially in those products with high n-3 PUFA concentrations, which produced higher concentrations of lipid degradation products.

Smell quality of all of the modified sausages obtained low scores in this parameter. As Murgueza et al. [17] have been reported; one of the limiting factors for introducing fish oil or derivatives into foodstuffs is the fishy smell that negatively affects sensory characteristics. Park et al. [33] detected undesirable flavor from fish oil when chicken frankfurters were prepared with 5 % fish oil. Murgueza et al. [34] concluded that sausages with a concentrated fish oil extract rich in n-3 fatty acids were not acceptable from the sensory point of view due to off odors. According to the panel, sausages S4, S5 and S6 obtained highest values in this parameter in comparison to controls C1 and C2 and the commercial brand. This fact could be due mainly to the oxidation of n-3 fatty acids. Oxidation of vegetables oils that have predominantly n-6 fatty acids will produce "grassy" and "beany" odors while oxidation of the long chain n-3 fatty acids in marine oils will produce 'fishy" odors.

As reported by Pelser et al. [8], no major differences were found in spicy intensity between the different samples, C1, S4 and S6, and C2 and S5, respectively. This was expected because the additions of salt and spices were equal for all the formulations; C1 showed the highest value of oily smell of all including the commercial brand. In general, the assessors did not find differences in texture between ostrich sausages and the commercial brand used as a standard. Comparing the sausages prepared with beef meat and ostrich meat, no matter the type of oil or fat used in the formulation, sausages prepared with ostrich meat can be resembled better in texture to the brands in the actual market than those prepared with beef meat. Only S7 differed in smell intensity. Nevertheless, the smell quality of all of the sausages was scored lower than the commercial standard. This confirms that all of the samples were in certain stage of oxidation.

After 60 days of storage, all the sausages had a lower score in their hedonic nature than the commercial brand. In comparison to the commercial brand, sausage C1 was ranged with the highest score in texture and color (9 points), that means that was no preferred for the sensory panel. These results can be compared with the instrumental measurement of color, which shows a loss of the color during storage, especially, in redness. The highest scores in odor were found in sausages S2 and S3 (10 and 9 points), panelists punctuated these sausages as none preferred due to the odor produced by lipid oxidation products. According to Fernández-López et al. [36], pigment and lipid oxidation are the major deteriorative reactions in meat and meat products during storage. The development of lipid oxidation products is responsible for a significant loss in quality characteristics such as color, flavor, texture and nutritive value [37].

5. Conclusions

Sausages prepared with ostrich meat instead of beef meat reduced the total fat content. Substitution of pork backfat by encapsulated oils as linseed, algal and fish oil, rich in n-3, reached important nutritional benefits. Sample S1 prepared with ostrich meat and encapsulated linseed oil, showed a fatty acid profile in which the P/S ratio was increased and at the same time the n-6/n-3 ratio was decreased. According to the results obtained the incorporation of ostrich meat and encapsulated linseed oil to this kind of product presents great advantages due to the increase in PUFAs and decrease the saturated fatty acid. However, this increase in PUFAs and the type of meat using in the preparation of Dutch style semi-dry fermented sausages adversely affected the preservation of the product during storage period due to the susceptibility of PUFAs to the lipid oxidation reactions, causing deterioration and a loss of quality. Despite, the new strategies needs to be developed in order to preserve the healthy advantages of these types of sausages, while at the same time avoiding reduced quality during storage and prolonging the shelf life.

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