

TITLE:

Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages.

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Abstract

Six formulations of dry fermented sausages were produced in three replications with three initial fat levels (30% , 20% and 10%) and two levels (0 and 20%) of replacing pork backfat with olive oil. After 4 weeks of fermentation and ripening the fat content of the treatments with 30%, 20% and 10% fat level ranged from 38.86 to 43.60%, 25.56 to 26.86% and 19.01 to 20.14% respectively. Fat level affected ($p<0.05$) the weight losses, the chemical composition, the Gram -ve bacterial count, the lightness, the texture and the appearance of fermented sausages. Replacing 20% of pork backfat by olive oil affected ($p<0.05$) the lightness and yellowness of sausages. Fat-reduced sausages without olive oil and low-fat sausages with olive oil had the highest score for odour and taste. However, the appearance of fat-reduced sausages was just acceptable while that of low-fat sausages was unacceptable, because the surface was intensively wrinkled and case hardening was developed. Further research is needed to improve the appearance of these sausages.

Key words: Fermented sausages, reduced fat, olive oil, colour, instrumental texture, sensory score.

1. Introduction

Health organizations all over the world have promoted lowering the intake of total dietary fat, particularly saturated fatty acids and cholesterol, as a mean of preventing cardiovascular heart disease (AHA, 1986; NCEP, 1988; Department of Health, 1994).

Dry fermented sausages are meat products with a high fat content, which is visible in the sliced product. Dry sausages made with a normal recipe have fat content around 32% directly after manufacture, but during the first week these values rises to about 40%, as a result of drying, and after 4 weeks to about 40-50% (Wirth, 1988). Fat in any food serves three basic physiological functions: a source of essential fatty acids, a carrier of fat soluble vitamins and as an energy source (Mela, 1990). In meat products fat contributes to the flavour, texture, mouth feel, juiciness and overall sensation of lubricity of the product. Therefore, any fat reduction can affect the acceptability of the products (Giese, 1996; Huffman & Egbert, 1990). Furthermore, the granulated fat in fermented sausages has important technological functions. It helps to loosen the sausage mixture and this aids the continuous release of moisture from the inner layers of the product; a process absolutely necessary for undisturbed fermentation and flavour/aroma development. For these reasons dry fermented sausages are the most difficult among meat products as far as fat reduction is concerned (Wirth, 1988).

Although there is a lot of research work for reduced and low-fat frankfurter type sausages, ground beef and hamburger, fresh pork and coarse ground sausages, there are only a few papers concerning fermented sausages (Giese, 1992; Keeton, 1994). According to Wirth (1988), if the climatic conditions are properly controlled during fermentation and drying fat-reduced salami and saveloy products of acceptable standard can be made with fat contents in raw material about 15%. Papadima & Bloukas (1999) found that in traditional Greek sausages the fat can be reduced to 20%. Recently, Mendoza, Garcia,

Casas & Selgas (2001) studied the effect of inulin, a soluble dietetic fibre, as a fat substitute in dry fermented sausages and found that acceptable low fat dry fermented sausages could be produced with approximately 40-50% of the fat content and 30% less total calories than conventional sausages.

Olive oil is the most monounsaturated vegetable oil, containing 56-87% monounsaturated fatty acids (MUFA), 8-25% saturated fatty acids (SFA) and 4-22% polyunsaturated fatty acids (PUFA) (IOOC, 1984). Olive oil has a high biological value, attributed to its high ratio of vitamin E to PUFA (Viola, 1970), lower ratio of SFA to MUFA than any other vegetable oil and antioxidant substances at an optimum concentration (Christakis, Fordyce & Kurtz, 1980). Finally, olive oil contains approximately 1% C₁₈ n-3 (alpha linolenic acid) which is a precursor for longer chain unsaturated omega-3 fatty acids such as EPA and DHA, normally only derived in the diet from oily fish (Kiritsakis, 1999). Prevalence of heart disease is relatively low in areas of the Mediterranean region where there is high consumption of olive oil (Keys et al., 1986; Aravanis & Dontas, 1978). In addition, Trichopoulou et al. (1995) found that increased olive oil consumption was associated with significantly reduced breast cancer risk whereas margarine intake appeared to be associate with an elevated risk of the disease. Finally, Roche, Gibney, Kafatos, Zampelas & Williams (2000) have shown that olive oil has very novel beneficial effects on postprandial lipid metabolism and thrombosis. Therefore, the positive effects for consumer health could be further improved by producing fermented sausages with the simultaneous reduction of fat level and partial replacement of pork backfat with olive oil. Bloukas, Paneras & Fournitzis (1997) have found that up to 20% of pork backfat can be replaced by olive oil in the form of pre-emulsified fat with soy protein isolate without negatively affecting the processing and quality characteristics of dry fermented sausages. Muguerza, Gimeno, Ansorena, Bloukas

& Astiasaran (2001) have also found that up to 25% of pork backfat can be replaced with pre-emulsified olive oil in the production of Chorizo de Pamplona fermented sausages, resulting in nutritional advantages related to cholesterol reduction and an increase in MUFA and PUFA fractions. However, no research has been done with fermented sausages concerning the simultaneous reduction of fat and the substitution of pork backfat with olive oil.

The objective of this study was to investigate the effect of fat reduction level and the substitution of 20% of pork backfat with olive oil in processing and quality characteristics of fermented sausages.

2. Material and methods

2.1. Sausage formulation and processing

Fresh boneless pork hams, fresh pork backfat and fresh boneless beef, cut from the shoulder, were obtained from a local supermarket. Pork and beef meat were trimmed of visible fat and pork backfat of adhering skin. Both meats and pork backfat were weighed in the appropriate amounts, vacuum packed and kept frozen at -20°C for at least one week. Representative samples were analyzed for moisture, fat and protein (AOAC, 1990) prior to freezing. All raw materials were tempered at -5°C for 24 h prior to use.

Commercial olive oil (Minerva Oil Processing Company, Athens, Greece) was obtained from a market. Olive oil was used as pre-emulsified fat (Hoogenkamp, 1989 a,b; Bloukas et al., 1997). Eight parts of hot water were mixed for 2 min with one part of isolated soy protein (ISP), type Pro-fam-974 (Archer Daniels Midland Company, Decatur, IL, USA) and the mixture was emulsified with 10 parts of olive oil for 3 min.

Six treatments of fermented sausages were prepared at three levels of fat content the day of preparation (30%, 20% and 10%) and two levels of replacing pork backfat with olive oil (0 and 20%). The experimental design and the formulation of raw materials is

given in Table 1. The following common ingredients were also added per kg of meat mixture in each treatment: sodium chloride, 28 g ; sodium nitrite, 0.2 g; sodium ascorbate, 0.5 g; sugar, 3 g; lactose, 1 g; white pepper, 3 g; garlic, 0.5 g; and starter culture, 0.25 g. Flora Carn SL 200 (Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark) containing *S. carnosus* and *L. pentosus*, was used as starter culture. Each treatment was produced at about 7 kg in three replications at different time periods and with different raw materials each time. The frozen beef and pork meat were cut and pre-weighed amounts of beef and pork meat were chopped for 2-3 sec in a Kilia 30 L cutter (Kilia Fleischermaschinenfabrik, Kiel, Germany) at low speed and mixed with all other ingredients, except sodium chloride. The pork backfat and the pre-emulsified fat were added and the meat mixture was chopped for 2-3 s; the cup of the cutter was cleaned, sodium chloride added and the meat mixture was chopped at low speed to the desired particle size, about 3-5 mm. Immediately after chopping, the prepared sausage mixture was stuffed using a Risco Breveti, model RS 3000 Baby, vacuum stuffer (Risco Breveti, Zane-vi, Italy) into 47 mm diameter Nojax collagen casings (Viscase SA, Bagnold Cedex, France). Sausages were handlinked to standard sizes (1.0-1.5 kg each), the resultant strings of sausages were placed for 30 days in the fermentation room where they remained under conditions similar to those applied in industry (Table 2). Samples from each treatment were taken for analysis on day 0, 3, 7, 15, 21 and 28.

2.2. Chemical analysis

Moisture, fat (ether-extractable), protein and ash were determined according to standard AOAC (1990) procedures. *pH measurement*: It was measured in a homogenate prepared by blending 20 g of sausage with 80 ml of distilled water for 30 s. Readings were taken with a WTW, model pH 521, digital pH meter and a WTW, type E56, combination electrode (WTW-Wissenschaftlich-Technische Werkstaetten GmbH,

Weilheim, Germany). *Weight losses*: Two strings of sausages from each treatment were weighed just before the fermented sausages were put into the fermentation room. The same strings were reweighed on day 3, 7, 15, 21 and 28. The differences were expressed as percentage of the initial weight. *Colour measurements*: They were taken with a HunterLab, model Labscan 5000, spectrophotometer (Hunter Associates Laboratory Inc, Reston, VA, USA) using a 10 mm port size, illuminant D₆₅ and a 10° standard observed. CIELAB L*, a* and b* values were determined as indicators of lightness, redness and yellowness. Four measurements were taken from each surface of two slices. Data presented are means of 16 measurements. *Firmness*: It was measured with a Zwick penetrometer (Rudolf Mueller & Co, Giessen, Germany) as described by Linhard and Liepe (1977). The higher the Zwick units, the firmer the sausages. *Hardness*: It was measured using the Stable Micro System Model TA.XT 2i (Stable Micro Systems, Godalming, England) texture analyzer. Three samples, 2 cm high and 2.5 cm in diameter, were used per treatment. The rate of deformation was 30% and the percentage of deformation 50%. Hardness was determined as the maximum force required to compress the sample and expressed as N/mm².

2.3. Microbiological analysis

A 20 g sample of sausages was homogenized in a Waring blender (Waring, New Hartford, CT, USA) with 180 ml sterile 0.1% peptone water for 2 min. Appropriate dilutions of samples were prepared in sterile 0.1% peptone water blank and plated in duplicate on different growth media. The following media and incubation conditions were used: (a) Man Rogosa Sarpe (MRS) Agar (Merck) at 30°C for 48-72 h, for lactic acid bacteria and (b) Nutrient (N) Agar at 20°C for 5 days for Gram -ve bacteria. These samples were taken on day 0, 7, 15 and 28 of production.

2.4. Sensory evaluation

Fermented sausages were evaluated 30 days after initial production by a seven-member sensory panel with previous experience judging fermented sausages. Two training sessions (about 30 min per session) were held in which commercial fermented sausages were used. Furthermore, a preparatory session was held before each panel to thoroughly discuss and clarify each attribute to be evaluated. Testing was initiated after the panelists agreed on the specifications. The evaluations were performed in individual booths under white fluorescent lights with the temperature of the product approximately ambient. Panelists were at first served with a half sausage from each treatment, cut across its axis, and asked to evaluate the appearance and the firmness according to the following scales: *Appearance* (determined by the visual appraisal based on the presence of wrinkles on the surface and case hardening at cross section): 7=smooth surface without wrinkles and cross section without case hardening, 1=wrinkled surface and intensive case hardening at cross section. *Firmness* (determined by the force required to induce with hands any deformation to the sausages): 7=very hard, 1=very soft. After that the panelists were served with six randomised slices of sausages, each per treatment, together with crackers and room temperature water to clean the palate between samples. The panelists were asked to evaluate the colour and the odour and taste of sausages according to the following scales. *Colour*: 7= too red, 1=totally discoloured (red to brown or to white). *Odour and taste*: 7=intensive pleasant odour and mildly sweet-acidic taste, 1=totally unpleasant odour and taste.

2.5. Statistical analysis.

Data collected for pH, weight losses, microbiological counts, colour and chemical composition were analyzed by a three factor factorial analysis, and for texture and sensory evaluation data by a two factor factorial analysis in a completely randomised design. The

factors were a) the three fat levels (30%, 20% and 10%), b) the two replacing levels of olive oil for pork backfat (0 and 20%) and c) the processing time for each parameter. Means were compared using the $LSD_{0.05}$ test. Data analysis were performed using the MSTAT (1985) program (Version 3.0, Michigan State University, East Lansing, MI, USA). Simple correlations were determined between selected response variables.

3. Results and discussion

3.1. Effect on processing characteristics

Neither fat level and nor the replacement of 20% pork backfat with olive oil had an effect on pH values whilst processing time had a significant effect ($p < 0.001$) (Table 3, Fig. 1a). The initial pH of fermented sausages ranged from 6.03 to 6.29 and the final pH on the 28th day from 4.70 to 4.92 (Fig. 1a). Bloukas et al. (1997) found a similar change of pH in fermented sausages with 25% fat level in which 0, 10 and 20% of pork backfat was replaced with olive oil either as liquid or as pre-emulsified fat (PEF) with soy protein isolate (SPI). Organic acids, mainly lactic acid, are formed in high acid fermented sausages as a result of carbohydrate breakdown during fermentation resulting in pH values below 5.3 (Incze, 1992).

Weight losses were significantly affected by the fat level ($p < 0.001$), the processing time ($p < 0.001$) and the interaction of total fat level and processing time ($p < 0.01$), but not by the inclusion of olive oil (Table 3). The higher the fat level the lower the weight losses over the same processing time (Fig. 1b) as also seen by Klettner et al. (1980), and Papadima and Bloukas (1999). Reducing the fat level of the meat mixture from 30% to 20% and 10% the required processing time for 30% weight losses is reduced from 28 to 15 and 9 days, respectively (Fig. 1b). Weight losses depend on many factors including the temperature and relative humidity of the ripening room, the air movement and the ripening time (Stiebing & Roedel, 1987 a,b), the degree of comminution of the meat

mixture and the width of the casings (Roedel & Klettner, 1981), the material of which the casings are made (Klettner & Roedel, 1980) and the fat content of sausages (Klettner et al., 1980).

3.2. Effect on chemical composition

The fat content of fermented sausages on the day of preparation was close to the targeted values (Table 4). Variations in the trimming of meats from visible fat in addition to the variations encountered during sampling may have contributed to the observed differences. Fat level and processing time significantly affected ($p < 0.05$) the chemical composition of fermented sausages whereas replacing some of the pork backfat with olive oil had no effect ($p > 0.05$). The higher the added fat level the lower the moisture and protein content and the higher the fat content. (Table 4). The differences between the treatments were significant ($p < 0.05$). The actual reduction in fat content was 30.88-39.08% for reduced fat and 48.14-56.39% for low-fat fermented sausages. No differences ($p > 0.05$) in fat content were found between the treatments of the same fat level with or without olive oil.

3.3. Effect on microbiological counts

Fat level and olive oil inclusion had no effect ($p > 0.05$) on lactic acid bacteria count (Table 3) which increased by about 2 log cycles during the first week and remained rather constant thereafter (Fig. 2a). A good correlation ($r = -0.927$, $p < 0.01$) was found between lactic acid bacteria count and pH, as also shown by Bloukas et al. (1997). The microflora of fermented sausage during fermentation and ripening changes as a result of the combined effects of lowering the pH, increasing the brine content and decreasing the water activity due to drying, and results in high populations of lactic acid bacteria (Palumbo, Zaika, Kissinger & Smith, 1976; Paneras & Bloukas, 1984; Luecke, 1986; Samelis, Aggelis & Metaxopoulos, 1993; Flores & Bermell, 1996). High populations of

lactic acid bacteria in fermented sausages inhibit the growth of spoilage and pathogenic bacteria, especially of *Staphylococcus aureus* (Geisen, Luecke & Kroeckel, 1992). In addition, it has been reported that lactic acid bacteria, taken with food, have a positive effect on human health (Fernades and Shahani, 1990; Incze, 1992).

Fat level and processing time significantly affected ($p < 0.001$) the Gram -ve bacteria count (Table 3). A significant effect on Gram -ve bacteria had also the interactions of fat level and processing time ($p < 0.05$) and olive oil replacing level and processing time ($p < 0.01$). Gram -ve bacteria are generally considered as an undesirable microflora in fermented sausages. Initial counts were around 5.0 log cfu/g, and reduced about 4 log cycles during ripening (Fig 2b). This decrease was inversely proportional to the increase in lactic acid bacteria as they suppress the growth of Gram -ve bacteria by producing organic acids and various antibacterial metabolic products (Daeschel, 1989; Weber, 1994; Holzapfel, Geisen & Schillinger, 1995).

3.4. *Effect on colour*

The influence of fat level, olive oil inclusion and processing time on colour is shown in Table 3. Both, higher fat level and inclusion of 20% olive oil produced lighter colour sausages (Table 3, Fig. 3a). The redness of sausages was only affected by processing time ($p < 0.001$) (Fig. 3b). However, the sensory evaluation scored low-fat sausages as being significantly ($p < 0.05$) redder than the others (Fig. 5a). Replacing 20% of pork backfat with olive oil produced more yellow sausages ($p < 0.001$) (Fig. 3c). Bloukas et al. (1997) also found that fermented sausages with 22-25% fat level in which 10% to 20% of pork backfat was replaced by olive oil were lighter and more yellow than controls. The yellowness of the sausages significantly reduced ($p < 0.001$) during processing time.

3.5. *Effect on texture*

Reducing the fat content of the sausages significantly increased ($p < 0.05$) instrumental hardness and firmness (Fig. 4). Hardness and weight loss were highly correlated ($r = 0.938$, $p < 0.01$). Replacing 20% of the pork backfat with olive oil had no effect ($p > 0.05$) on instrumental hardness or firmness, as was also seen by Bloukas et al. (1997). The sensory evaluation showed similar results for firmness (Fig. 4c). Sensory firmness and weight loss were also highly correlated ($r = 0.99$, $p < 0.01$)

3.6. Effect on odour and taste and appearance

Fat level and olive oil replacement affected ($p < 0.05$) the sensory odour and taste of sausages. Reduced-fat fermented sausages without olive oil and low-fat sausages with olive oil had the highest scores for odour and taste (Fig. 5b).

Total fat level significantly affected ($p < 0.05$) the appearance of fermented sausages while the replacing 20% of pork backfat with olive oil had no effect ($p > 0.05$). The lower the fat level the lower the scores for appearance (Fig. 5c). Low-fat fermented sausages with or without olive oil had more intensively wrinkled surface and more intensive case hardening at cross section, probably due to higher weight losses. The appearance score for these fermented sausages was on the 30th day even lower than the limit of acceptability.

4. Conclusions

Reduced and low-fat fermented sausages can be prepared with 20% and 10% fat in the meat mixture, respectively, and 20% of the added pork backfat can be replaced with olive oil pre-emulsified with soy protein isolate. The finished products will have a fat content about 27% and 20%, respectively, which will be about 37% and 53% lower than a regular high fat control sausage (30% fat). The reduction of fat level increases the weight losses, the hardness and firmness of sausages and makes them darker and redder. The replacement of 20% pork backfat with olive oil does not affect the weight losses but

makes the sausage colour lighter and more yellow. Although these products have acceptable odour and taste, low-fat fermented sausages with or without olive oil, have an unacceptable appearance, due to intensively wrinkled surface and the development of case hardening. Therefore, further research is needed to improve the sensory attributes of these products and particularly their appearance.

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References

- AHA, (1986). Dietary guidelines for healthy adult Americans. *American Heart Association 74 (circulation)*, 1465-1475 A.
- AOAC. (1990). *Official method of analysis*. Association of Official Analytical Chemist. Washington, D.C.
- Aravanis, C. & Dontas, A. (1978). Seventeen-year mortality from coronary heart disease in the Greek islands heart study. *Abstracts, 18th Annual. Conference on Cardiovascular Disease Epidemiology*. Orlando, FL.
- Bloukas, J.G., Paneras, E.D. & Fournitzis, G.C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, 45 (2) 133-144.
- Christakis, G., Fordyce, M.K., & Kurtz, C.S. (1980). The biological aspects of olive oil. *In proceedings of 3rd international congress on the biological value of olive oil*. Hania, Greece.
- Daeschel, M.A. (1989). Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology*, 43 (1), 164-166.

- Department of Health. (1994). Report on health and social subjects, n° 46. *Nutritional aspects of cardiovascular disease*. London: HMSO.
- Fernades, C. F., & Shahani, K.M. (1990). Anticarcinogenic and immunological properties of dietary lactobacilli. *Journal of Food Protection* 53, 704-713.
- Flores, J., Bermell, S. (1996). Dry-cure sausages-factors influencing souring and their consequences. *Fleischwirtschaft*, 76 (2), 163-165.
- Geisen, R., Luecke, F.K., & Kroeckel, L. (1992). Starter and protective cultures for meat and meat products. *Fleischwirtschaft*, 72 (6), 898-984.
- Giese, J. (1992). Developing low-fat meat products. *Food Technology*, 46 (4), 100-108.
- Giese, J. (1996). Fats, oils and fat replacers. *Food Technology*, 50 (4), 78-83.
- Holzappel, W.H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology* 24, 343-362.
- Hoogenkamp, H.W. (1989 a). Low-fat and low-cholesterol sausages. *Fleischerei*, 40 (10), III-IV.
- Hoogenkamp, H.W. (1989 b). Low- calorie sausages, spreads and mousses. *Fleischerei*, 40 (11), IV-V, (12), III-IV.
- Huffman, D.L., & Egbert, W.R. (1990). Chemical analysis and sensory evaluation of the developed lean ground beef products. In *Advances in Lean Ground Beef Production*. Alabama Agriculture. Ex. Sta. Bull. No 606. Auburn University, Alabama, USA.
- Incze, K. (1992). Raw fermented and dried meat products. *Fleischwirtschaft*, 72 (1), 58-62.
- IOOC (International Olive Oil Council) (1984). *International trade standards applying to olive oil and olive residue oils*. COI/T. ISNC No 1.

- Keeton, J.T. (1994). Low-fat meat products- technological problems with processing. *Meat Science* 36, 261-276.
- Keys, A., Menotti, A., Karvonen, J.M., Aravanis, C., Blackburn, H., Buzina, R., Djorjevic, B.S., Dontas, A., Fidanza, F., Keys, H.M., Kromhout, D., Nedeljkovic, S., Punsar, S., Seccareccia, F. & Toshima, H. (1986). The diet and 15-year death rate in the seven countries study. *American Journal of Epidemiology* 124, 903-918.
- Kiritsakis, A.K. (1999). Olive oil. From the tree to the table. *Food and Nutrition Press, Inc.*, Trumbull, CT.
- Kletnner, P.G., Roedel, W. (1980). Beitrag zum Einfluss des Speckanteils auf die Rohwurstreifung. *Fleischerei* 11, 1101-1104.
- Linhard, O.A. & Liepe, H.U. (1977). Festigkeitsmessung an Rohwürsten mittels eines Hartepressgerätes. *Die Fleischwirtschaft* 57, 1235-1238.
- Luecke, F.K. (1986). Microbiological processes in the manufacture of dry sausage and raw ham. *Fleischwirtschaft*, 66 (10), 1505-1509.
- Mela, D.J. (1990). The basis of dietary fat preference. *Trends in Food Science and Technology*, 1 (13), 55-78.
- Mendoza, E., Garcia, M.L., Casas, C., & Selgas, M.D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science* 57, 387-393.
- MSTAT. (1985). *Design and analysis of agronomic research*. Michigan State University: East Lansing, MI.
- Muguerza, E., Gimeno, O., Ansorena, D., Bloukas, J.G., & Astiasaran, I. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of Chorizo de Pamplona-a traditional Spanish fermented sausage. *Meat Science*, 59, 251-258. .

- NCEP (National Cholesterol Education Program). (1988). The effect of diet on plasma lipids, lipoproteins and coronary heart disease. *Journal of American Diet Association* 88, 1373-1400.
- Palumbo, S.A., Zaika, L.K., Kissinger, J.G., Smith, J.L. (1976). Microbiology and technology of the pepperoni process. *Journal of Food Science* 41, 12-17.
- Paneras, E.D., & Bloukas, J.G. (1984). A study of commercial fermented sausages production using natural fermentation, starter cultures and glucono-delta-lactone. *In proceeding of 30th European Meeting of Meat Research Workers* (pp. 344-347). Bristol,
- Papadima, S.N., & Bloukas, J.G. (1999). Effects of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science* 51, 103-113.
- Roche, H.M., Gibney, M.J., Kafatos, A., Zampelas, A., & Williams, C.M. (2000). Beneficial properties of olive oil. *Food Research International* 33, 227-231.
- Roedel, W., Klettner, P.G. (1981). Beitrag zum Einfluss des Huellenkalibers auf die Rohwurstreifung. *Fleischerei* 32, 803-806.
- Samelis, J., Aggelis, G.L., Metaxopoulos, J. (1993). Lipolytic and microbial changes during the natural fermentation and ripening of Greek dry sausages. *Meat Science* 35, 1-15.
- Stiebing, A., Roedel, W. (1987a). Einfluss der Luftgeschwindigkeit auf den Reifungsverlauf bei Rohwurst. *Fleischwirtschaft* 67, 236-240.
- Stiebing, A., Roedel, W. (1987b). Einfluss der relativen Luftgeschwindigkeit auf den Reifungsverlauf bei Rohwurst. *Fleischwirtschaft* 67, 1020-1030.
- Trichopoulou, A., Katsouyanni, K., Sturer, S. Tzala, L., Gnardellis, Ch., Rimm, E. & Trichopoulou, D. (1995). Consumption of olive oil and specific food groups in

relation to breast cancer risk in Greece. *Journal National Cancer Institute* 87, (2), 110-117.

Viola, P. (1970). Fats in the Human Diet. Olive oil. *International Olive Oil Council*, (Ed.). Madrid, Spain.

Weber, H. (1994). Dry sausage manufacture-the importance of protective cultures and their metabolic products. *Fleischwirtschaft* 74, 278-281.

Wirth, F. (1988). Technologies for making fat-reduce meat products. What possibilities are there?. *Fleisechwirtschaft*, 68 (9), 1153-1156.

Fig. 1. Effect of fat level and olive oil inclusion on (a) pH and (b) weight losses during fermentation and ripening of fermented sausages.

-: HF; ☐-☐ : RF; Δ-Δ: LF; ✕- - -✕: HFO; ☐ --- ☐ : RFO; ▲---▲: LFO.

Fig. 2. Effect of fat level and olive oil inclusion on (a) Lactid acid bacteria and (b) Gram -ve bacteria during fermentation and ripening of fermented sausages.

-: HF; ☐-☐ : RF; Δ-Δ: LF; ✕- - -✕: HFO; ☐ --- ☐ : RFO; ▲---▲: LFO.

Fig.3. Effect of fat level and olive oil inclusion on (a) Lightness (L*), (b) Redness (a*) and (c) Yellowness (b*) during fermentation and ripening of fermented sausages.

-: HF; ☐-☐ : RF; Δ-Δ: LF; ✕- - -✕: HFO; ☐ --- ☐ : RFO; ▲---▲: LFO.

Fig. 4. Effect of fat level and olive oil inclusion on (a) instrumental hardness (N/mm²), (b) instrumental firmness (Zwicks Units) and (c) sensory firmness of ripened fermented sausages. ^{a-c}Treatments with different superscript letters are significantly different for the respective attribute (p<0.05).

Fig. 5. Effect of fat level and olive oil inclusion on (a) sensory colour, (b) odour and taste and (c) appearance of ripened fermented sausages. ^{a-c}Treatments with different superscript letters are significantly different for the respective attribute (p<0.05).

Table 1

Experimented design and raw materials used

Treatments*	Fat	Olive oil	Materials in g/kg of meat mixture					
	level ^a (%)	replacing level (%)	Beef meat	Pork meat	Pork backfat	Olive oil	ISP ^b	Water
HF	30	0	220	440	340	-	-	-
RF	20	0	260	520	220	-	-	-
LF	10	0	300	600	100	-	-	-
HFO	30	20	200	400	272	68	6.8	54.4
RFO	20	20	250	490	176	44	4.4	35.2
LFO	10	20	290	590	80	20	2.0	16

*HF=High fat (all animal fat), RF=Reduced fat (all animal fat), LF=Low fat (all animal fat), HFO=High fat with olive oil, RFO=Reduce fat with olive oil, LFO=Low fat with olive oil. ^a On the day of preparation. ^b ISP=Isolated soy protein

Table 2

Processing conditions for fermented sausages

Day	Temperature (° C)	Relative humidity (%)	Air movement (m/sec)	Smoking
Fermentation				
0	20.5	95	0.5-0.7	
1	20	93	0.5-0.7	
2	19	90	0.5-0.7	
3	18	88	0.5-0.7	
4	17	85	0.5-0.7	
5	15	80	0.5-0.7	+
Ripening				
6-30	15	80	0.05-0.1	

Table 3

Analysis of variance on the effect of total fat and olive oil replacing level for pork backfat on physico-chemical and microbiological parameters of fermented sausages (*F*-values of independent variables and interactions)

Parameter	Source of variance						
	(A)	(B)	(C)	AxB	AxC	BxC	AxBXC
Processing parameters							
pH	0.05ns	0.48ns	14.38***	0.12ns	0.07ns	0.15ns	0.10ns
Weight loss	38.52***	0.20ns	201.86***	0.21ns	0.02**	0.04ns	0.19ns
Microbiological counts							
Lactic acid bacteria	1.01ns	2.35ns	28.96***	0.42ns	0.15ns	0.26ns	0.27ns
Gram -ve bacteria	26.51***	2.09ns	1009.38***	2.46ns	2.97*	3.31**	1.29ns
Colour							
Lightness (L*)	7.46**	10.91**	2.50ns	0.64ns	0.08ns	0.81ns	0.43ns
Redness (a*)	0.60ns	0.07ns	6.19***	0.54ns	0.32ns	0.35ns	0.26ns
Yellowness (b*)	0.08ns	2.69*	9.22***	0.03ns	0.06ns	0.32ns	0.08ns

A = Total fat level, B = Olive oil replacing level for pork backfat, C = Processing time

* Significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$; ns = not significant

Table 4

Effect of total fat and olive oil replacing level for pork backfat on chemical composition of fermented sausages

Treatment*	Moisture %	Protein %	Fat %	Ash %
0 Day				
HF	54.35 ^c	14.27 ^g	28.95 ^b	2.3 ^b
RF	58.54 ^b	18.72 ^{ef}	20.17 ^{cd}	2.57 ^{ab}
LF	64.47 ^a	19.87 ^e	12.97 ^e	2.69 ^{ab}
HFO	52.31 ^c	15.80 ^{fg}	29.34 ^b	2.55 ^{ab}
RFO	58.46 ^b	18.54 ^{ef}	20.43 ^{cd}	2.57 ^{ab}
LFO	64.30 ^a	19.95 ^e	13.03 ^e	2.72 ^{ab}
28 Day				
HF	26.08 ^f	26.42 ^d	43.60 ^a	3.90 ^{ab}
RF	35.37 ^d	33.62 ^{bc}	26.56 ^b	4.45 ^{ab}
LF	37.19 ^d	38.5 ^a	19.01 ^d	5.33 ^{ab}
HFO	30.98 ^e	25.99 ^d	38.86 ^a	4.17 ^{ab}
RFO	35.06 ^d	33.15 ^c	26.86 ^b	4.93 ^{ab}
LFO	37.57 ^d	37.67 ^{ab}	20.14 ^{cd}	5.61 ^a
Processing time**				
0 Day	58.24 ^b	17.86 ^{ef}	20.81 ^{cd}	2.59 ^{ab}
28 Day	33.70 ^d	32.69 ^c	30.05 ^b	4.66 ^{ab}
L.S.D. _{.05}	3.17	2.99	5.39	3.13

* HF=High fat (all animal fat), RF=Reduced fat (all animal fat), LF=Low fat (all animal fat), HFO=High fat with olive oil, RFO=Reduce fat with olive oil, LFO=Low fat with olive oil.

** Each number of the table represents the average value of each parameter for all fermented sausages at the same time.

^{a-g} Means within same column with different superscript letters are significantly different. (p<0.05).