TeRiFiQ

Project no. 289397

Combining Technologies to achieve significant binary Reductions in Sodium, Fat and Sugar content in everyday foods whilst optimizing their nutritional Quality

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Deliverable D2.3 – Definition of new formulation and process conditions to develop dry-fermented sausages

Abstract: *This deliverable consists of a report that describes a number of technological paths overcoming the changes in mass transfers and aroma production mechanisms promoted by a drastic reduction in fat and salt during dry-fermented sausage manufacture.*

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RE Restricted to a group specified by the consortium (including the Commission Services)	[ ]
CO Confidential, only for members of the consortium (including the Commission Services)	[ ]

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Glossary

8O: Eight-way Olfactometry

a_w: Water activity

DFS: Dry-fermented sausage(s)

DHS: Dynamic headspace sampling

DNPH: Dinitrophenylhydrazine

DTNP: Dithiobis-itropyridine

GC: Gas chromatography

HCA: Hierarchical cluster analysis

HSB: Hydrosoluble Schiff base

KCl: Potassium chloride

LAB: Lactic acid bacteria

MDA: Malondialdehyde

MS: Mass spectrometry

NaCl: Sodium chloride (or salt)

NaOH: Sodium hydroxide

PI: Proteolysis index

PUFA: Polyunsaturated fatty acid

RH: Relative humidity

SD: Standard deviation

SFA: Saturated fatty acids

SFO: Sunflower oil

SPME: Solid phase microextraction

TBARS: Thiobarbituric acid reactive substances

TM: Total matter

toF: time of flight

TPA: Texture profile analysis

1. Summary

1.1 Objectives and approach engaged

With the focus on DFS, the main objectives of WP2 are to develop procedures allowing the production of safe, nutritionally improved products that are acceptable to consumers. The binary reduction goal targeted for DFS is a 30% reduction in sodium and a 60% reduction in SFA. In this context, the planned objective of Task 2.3 is to investigate, at lab scale, the impact of reducing animal fat and salt contents on water and salt transfers and the formation of odour and flavour compounds. Indeed, removing sodium and fat from DFS will alter the inner biochemical reactions (proteolysis, fermentation, oxidations...) due to changing water activity (a_w), and thus the final product aroma, flavour and taste.

The concrete objective set for Task 2.3 was to run two series of DFS manufacture to bring accurate data on the following three points: (1) the potential application of new technologies in DFS production (adding KCl as a substitute for NaCl, adding vegetable oil...) to lower SFA and sodium content, (2) the impact of fat and salt reduction on water and salt transfers and the formation of odour and flavour compounds, and (3) the product quality and consumer acceptability of low-sodium and low-fat DFS.

It was decided early on to uncouple the experimental study of water transfers from the study of aroma production and to complete these studies by a time–course study (on Day 1, D7, D21, and D29) to track and trend key biochemical parameters such as pH, proteolysis, fermentation and protein and lipid oxidations during the fabrication of DFS containing different amounts of sodium and animal fat. Since these biochemical analyses are fully destructive and need several repetitions, large numbers of DFS had to be fabricated and dried for about one month. For practical reasons, we thus used the pilot ripening rooms of our partner ADIV to manufacture these DFS instead of our laboratory's purpose-built micro-bioreactors (MBRA) in which only one DFS can be manufactured at a time. In total, two series of DFS were manufactured, representing 15 different batches of about 30 units per batch. The first series was designed to investigate the effect of reducing NaCl and animal fat content on the time–course of physicochemical parameters such as pH, weight loss and a_w and biochemical parameters such as proteolysis, lipolysis, fermentation, protein oxidation and and lipid oxidation. For the 8 batches corresponding to this first series, the animal fat and sodium content of each batch was fixed by building a Doehlert experimental design with two factors. In parallel, trials were carried out in which the ultimate drying stage was performed in our lab MBRAs. In the second series, the 7 batches for manufacture were chosen based on input from Task 2.1 on the beneficial use of new strategies (adding KCl and/or vegetable oil) to reduce sodium and SFA content as reported in deliverable D2.1. For all DFS batches, quality was assessed by texture profile analysis (TPA) tests, and in the specific case of the second series manufactured, by sensory descriptive analysis and consumer acceptability testing by a panel of 29 assessors accustomed to eating DFS.

In addition, while waiting for all the DFS to be manufactured, we led a special study to better understand the biochemistry of the aroma of DFS by identifying the odour-active molecules and their respective biochemical origins (from meat or fat, from flavouring, from unknown origins) using gas chromatography–olfactometry. These analyses were performed on products purchased commercially from producers identified by the laboratory in previous projects as manufacturing typical products presenting a sharp aroma of DFS. The same type of aroma analysis was also performed on DFS manufactured in the second series of fabrication, with the particular—but challenging—objective of finding interrelationships between aroma production, biochemical evolution and sensory acceptability by consumers.

1.2 Main results

1.2.1 Analysis of water and salt transfers

DFS drying globally leads to a reduction in in-DFS water content due to water evaporation from the DFS surface and, in turn, to a fat and salt concentration that increases fat and salt content, respectively. We therefore ran chemical analysis of water content, salt content and fat content at four timepoints, i.e. Day 1, Day 7, Day 14 and Day 29, to track and trend the time-course of these parameters and check, at the end of drying, whether the objectives of reducing salt by 30% and fat by 60% are effectively achieved.

For the two fabrication series, the respective proportions of lean meat and fat logically influence the in-DFS water content values. For an identical drying process, the highest water content values expressed in percentages are obtained for the low-fat products. The measured salt content values (NaCl and KCl) at Day 1 were still higher than the intended values, probably as a result of the natural presence of sodium, chloride and potassium ions in lean pork meat and very probably as a result of real difficulties in perfectly adjusting the amount of salt added during the meat batter preparation as a function of the respective proportions of lean pork meat and fat. However, the formulations with the higher intended salt content values nevertheless contained more salt (NaCl and KCl) than the others, once the meat batters were prepared, thus *a priori* lowering the impact of this observed discrepancy on the results subsequently obtained. In a similar way to salt content, there were discrepancies in total lipid content reaching 2% at most, again underlining the real difficulty in perfectly adjusting the amount of added fat during meat batter preparation. However fortunately, the formulations with the higher intended fat content values really contained more fat than the others, once the meat batters were prepared.

Time-course pattern of physicochemical parameters measured for DFS of the two fabrication series, namely DFS weight loss, mean in-DFS a_w and mean in-DFS pH values, was assessed. To make the results easier to interpret and the figures easier to read, a specific statistical treatment called hierarchical cluster analysis (HCA) was applied to all the measured raw values. HCA consists in clustering DFS formulations that lead to similar results on a given parameter, thereby creating classes of formulations. Concerning DFS water loss, the present results based on HCA analysis showed a strong impact of animal fat content whatever the fabrication series, with about 8% variation in DFS water loss according to the formulations, and a moderate effect of salt content, exclusively for the first DFS fabrication series. Concerning DFS water loss, whatever the fabrication series, the HCA analysis-based results presented here logically showed a strong impact of salt content, an impact of type of salt (NaCl or KCl), and also an effect of fat content. Indeed, modifying the fat content of the meat batter modifies the salt concentration in the lean part of the batter, and thus the a_w value. From an a_w perspective, reducing fat content in DFS provokes the same increase on a_w as reducing salt content. So, binary reductions in DFS fat and salt content may prove detrimental from a safety standpoint if the products are not sufficiently dried. Concerning in-DFS pH value, whatever the fabrication series, the HCA analysis-based results showed normal time-course of pH value, with two distinct phases: strong acidification during the first week of process followed by a progressive increase in pH value, except in the non-flavoured formulation. This therefore highlights a strong impact of flavouring on time-course of pH values, an impact of salt content, and a moderate effect of fat content. HCA analysis found no discernible effect of type of salt (NaCl or KCl).

The main conclusion from the MBRA drying trials is that surface microbial flora growth did not modify water transfers inside and at the surface of the product, in contrast to what had recently been observed on two types of cheese. This means that the water transfers (evaporated water flux, a_w and weight loss) in the dried products investigated here were directly induced by the drying conditions applied. That underlines the value of building a numerical function making it possible to calculate local a_w value as a function of local water content, local NaCl content and local fat content anywhere in a DFS. A DFS sorption isotherm was therefore built.

1.2.2 Time course of DFS biochemical parameters

Time-course of the biochemical parameters was also measured for DFS in the two fabrication series, namely proteolysis, lipolysis, lactic acid content, lipid oxidation and protein oxidation. To make the results easier to interpret and the figures easier to read, we ran a HCA on all the measured raw values.

The results confirmed that proteolysis in DFS was mainly governed by their salt content. Reducing the salt content increases proteolysis, which can be detrimental for the final texture of the end-products. This critical point warrants checking via the texture profile analysis on the DFS samples. Concerning lipolysis, the data seems to suggest that the intensity of this biochemical phenomenon is mainly dependent on fat content, but further quantification of lipolysis is needed, maybe using another experimental method than determination of acid value, to definitively conclude on the effect of reducing salt and fat content on DFS lipolysis. Concerning lactic acid content, acid production rates fit perfectly with pH time-course values. Concerning lipid oxidation, only the HSB quantification method showed that lipid oxidation increased with time. HCA-based results highlighted that lipid oxidation was more intense for the formulations containing either '21% animal fat' or '7% animal fat plus 3% SFO'. Therefore, care is warranted when using vegetable oil because this type of oil is very sensitive to lipid oxidation due to its high PUFA content. Protein and lipid oxidations are linked by the fact that lipid oxidation produces free radicals that, in turn, drive protein oxidation. Therefore, maximal protein oxidation occurred in high-fat formulations. The adding of SFO seems to also promote protein oxidation. Whatever the experimental quantification method used (carbonyl group content or free thiol group content), we found no clear change in protein oxidation with time. It would seem that protein oxidation occurs rapidly, maybe directly during the meat batter preparation, and without subsequent intensification.

1.2.3 DFS aroma analysis

We identified the odour-active compounds responsible for the characteristic aroma of DFS in order to find the simplest way to enhance the aroma of new low-fat low-salt products. To this end, we implemented powerful methods of identification using high-resolution gas-phase chromatography and mass spectrometry coupled with single- or multi-way olfactometry. The structural identification and odour characteristics of the key compounds in the aroma will enable us to trace their most likely origins (meat biochemistry, flavouring, etc.).

The odour-active compounds were identified on commercially available DFS, selected for their intense aroma. The results obtained indicate that the two main origins of the aroma of dry sausages can be assigned to (i) odour-active compounds forming during the degradation of animal tissues over the course of the fermentation and drying processes, and (ii) flavouring with natural substances. Given that the manufacturing recipes used by ADIV are widely recognized as industry-standard practice and that it is extremely difficult

to steer aroma in salt-cured products by acting solely on the fermentation processes, we elected to enhance the aroma of low-salt low-fat sausages by adding flavouring with natural substances. The results of gas-phase chromatography coupled with olfactometry prompted us to preferentially introduce odour-active compounds with “meaty” or “dry-cured” notes, which the assessors considered typical of cured products. The simplest way to do this was to flavour our experimental low-salt low-fat products mainly with a garlic-based extract. ADIV thus chose a powdered dried garlic extract that was easier to add and blend into the mixture. Black pepper was also added in the formulations. These two ingredients are additives already used separately or jointly in French manufacture of traditional products, and so their use in low-salt low-fat products should not surprise consumers. Moreover, the profiling of odour-active compounds identified in the DFS shows that the production of compounds formed by lipid oxidation depends largely on in-sausage fat level. Replacing NaCl by KCl proved to have limited effect on volatile biochemical markers.

1.2.4 Evaluation of DFS quality and consumer acceptance

DFS quality was first evaluated objectively through texture measurements by Texture profile Analysis test performed on non-frozen 30×20×50 mm parallelepiped samples extracted from 29-day-old DFS.

For the first fabrication series, global analysis of texture measurements indicated that:

- Regarding hardness, the highest values were obtained for the 3 low-fat fabrications, thus highlighting a highly significant effect of fat content. These high values were probably due to higher water loss of the DFS during drying. On the other hand, statistical analysis did not find any significant effect of NaCl content on DFS hardness.
- Regarding fragility, we found no significant difference between the 8 experiments on this textural parameter.
- Regarding cohesiveness, the lowest values were obtained for the two formulations containing only 2% NaCl, thus highlighting a highly significant effect of salt content on final product cohesiveness. These low cohesiveness values probably result from more intense proteolysis. On the other hand, we found no significant effect of animal fat content on product cohesiveness.
- Regarding elasticity, statistical analysis indicated that animal fat content has a highly significant effect on elasticity value, unlike salt content.

For the second fabrication series, global analysis of texture measurements showed that:

- Regarding hardness, the highest values were obtained for the 7%-animal fat formulations compared to the 21%-animal fat formulations, thus again highlighting a highly significant effect of fat content on final DFS hardness. Also, adding vegetable oil clearly modified DFS texture, making them harder. A very limited impact of using KCl was found on DFS texture. On the other hand, not adding flavouring was detrimental to final DFS texture, probably due to under-acidification during the fermentation stage. Finally, statistical analysis found no significant effect of NaCl or KCl content on DFS hardness.
- Regarding fragility, the 7 experiments did not differ significantly on this textural parameter.
- Regarding cohesiveness, statistical analysis found a significant effect of fat content but no significant effect of added vegetable oil, salt content or type of salt on final product cohesiveness.

- Regarding elasticity, statistical analysis indicated a highly significant effect of the animal fat content on elasticity value. There was no visible or statistically significant effect of salt content, type of salt or adding SFO on DFS elasticity.

DFS quality was also evaluated through a sensory analysis performed by 29 assessors with the objective of comparing consumer-panel acceptability of flavoured and non-flavoured low-salt and low-fat products. The results clearly show that salt and fat contents may be greatly reduced with no adverse effect on the acceptability of DFS: most of the low-fat low-salt flavoured products presented practically the same acceptability as full-fat full-salt reference DFS, even though their organoleptic characteristics were different. The role of flavouring proved very important, as it acted not only through the introduction of aromatic substances that enhance the acceptability of the aroma but also by activating fermentation processes that further shape texture acceptability. Flavouring, mainly with garlic, is one possible solution that we can advocate, since garlic has a long history use in French dry-cured meat products. Ultimately, various flavouring solutions will probably have to be implemented according to consumer tastes and eating habits in the countries or regions concerned in order to optimize the acceptability of new low-fat low-salt products. This is something that will be tested under WP6 in the case of Spanish chorizo, via a collaboration with the ADIV and the industry manufacturer Boadas 1880 S.A.

2. Introduction

2.1 Background

2.1.1 Brief bibliography

Excessive salt consumption is harmful to human health, yet NaCl also possesses many technological functions that make it a key ingredient in the production of dried-cured pork products (Weiss et al., 2010). Sodium chloride is primarily a preservative that protects a large variety of food systems against microbiological spoilage and/or undesirable or pathogenic microorganisms. Second, salt helps lend dried-cured pork products their characteristic flavour, colour and aroma. Third, it plays a decisive role in the final texture of the products, through mechanisms such as its action on the solubilization of meat myofibrillar proteins. In fact, salt preserves food products from all microbiological spoilage (Leistner, 1985) by lowering the water activity (a_w) to create a barrier effect. In the specific case of DFS manufacture, the salting step is followed by a fermentation step that causes a drop in pH and by a drying and ripening step that further reduces in-product water activity. The likely adverse effects of reducing sodium chloride content in dried-cured pork products include poor texture due to intense proteolysis, poor cohesiveness, which becomes a problem when the meats are sliced, and a reduction in the typical flavour and aroma of these products (Benedini et al., 2012). Several strategies can be used to reduce the sodium content of dried-cured pork products. The simplest is to directly and gradually reduce the quantity of sodium added when the products are being manufactured (He & Macgregor, 2009). However, studies tend to find that it is very difficult to reduce sodium content by more than 25% simply by lowering the amount of sodium chloride added during manufacture without adversely affecting product texture and/or aroma. In addition, according to Ruusunen and Puolanne (2005), in dried-fermented products, sodium chloride content cannot be simply reduced because a low a_w value has to be reached in order to ensure the products remain microbiologically stable. Another widely applied strategy is to replace some of the sodium chloride (NaCl) by substitute salts, particularly potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$) or potassium lactate ($C_3H_5KO_3$), which makes it possible to reduce overall sodium content while controlling a_w inside the products. The substitute salt most often used is KCl due to its similar behaviour in terms of protein solubilization and inhibition of protease activity (Armenteros et al., 2009). However, at high concentrations, KCl can generate a strong bitter and metallic taste in products. For example, Gou et al. (1996) highlighted unwanted bitterness in DFS once degree of substitution of NaCl by KCl reached 30%, although this defect remained acceptable up to a substitution rate of 40–50%. The main defects generated using substitute salts can be corrected by adding taste enhancers or masking agents, many types of which are commercially available (Desmond, 2006).

As a result of drying, pork products like DFS or salami can ultimately contain 30–50% animal fat which is a major determinant of the final sensory characteristics, i.e. flavour, texture, juiciness and appearance. However, excessive fat consumption is associated with increased risks of obesity, cancer, high blood cholesterol and coronary diseases. Besides the quantity of fat consumed, its qualitative composition also has strong impacts on human health. For example, ensuring cardiovascular health demands a very low consumption of trans fatty acids (less than 1%) and a sufficient supply of polyunsaturated fatty acids (PUFAs; 6–10%) in the daily energy intake, with the further constraint that these PUFAs should be well balanced between ω-6 (5–8%) and ω-3 (1–2%) at a ratio of 1–4 (Nishida et al., 2004). Reducing the animal fat content of DFS may require reformulation of the product. A product composed of lean meat, vegetable instead of animal fat, and other fat substitutes,

produced in appropriate manufacturing conditions, can help modify lipid profile and fat concentration (Weiss et al., 2010). Simply reducing the animal fat content predictably leads to a loss of aroma, which is often not readily accepted by the consumer. In experiments on Spanish DFS in which fat content ranged between 10% and 30%, Olivares et al. (2011) found that the reduction in fat slowed lipolysis, lipid oxidation and the ensuing formation of volatile compounds. Their results also showed that consumer acceptance was closely correlated with high fat levels and long ripening times. An earlier study by Olivares et al. (2010) had found that the limit between consumer acceptability and rejection of fat-reduced DFS corresponded to an initial fat content of 16%.

A binary reduction of the sodium and animal fat contents of dry-fermented pork meat products manifestly helps improve their nutritional value, but there have been scarce few studies combining sodium and animal fat reduction in dry-cured pork products, doubtless because these two reductions together cause a very marked loss of the typical aroma and taste expected of such products. Nevertheless, Beriain et al. (2011) designed research to quantify the effect of replacing half the pork fat by an emulsion of water, olive oil and alginate, incorporating inulin, and substituting 58% of the NaCl by 20% KCl and 38% $CaCl_2$ on the qualities of Pamplona chorizo. Trials showed that the incorporation of olive oil in an emulsion, associated with a 58% reduction in NaCl had no negative effect on manufacturing process technology, with no abnormal difference in time-course of pH or microbiological populations being detected at any time during manufacture. Chorizos containing alginate were shinier and harder than traditional ones, except for those also containing 6% inulin. In conclusion, Beriain et al's (2011) work showed that incorporating an emulsion based on alginate and olive oil with added inulin made it possible to manufacture Pamplona chorizos with less salt and less fat but more unsaturated fatty acids, thereby offering products with a better nutritional profile than those manufactured traditionally.

2.1.2 Main findings of Deliverable D2.1

One of the main objectives of the TeRiFiQ project is to reduce sodium and saturated fatty acid (SFA) content by 30% and 60%, respectively, from the average composition of French DFS measured as 1.82% sodium and 36.4% lipids with 14.6% SFA (Oqali, 2009). Deliverable D2.1 was dedicated to assessing the feasibility of using multiple emulsions, cryo-crystallized fats and pre-drying technologies in DFS production in order to reduce the sodium and animal fat levels in these products.

To reduce sodium content by 30%, three different technologies were tested in Task 2.1 and reported in Deliverable D2.1: (i) pre-drying meat before meat batter preparation, (ii) pre-drying sausages at cold temperature (8°C) before the fermentation stage, and (iii) partial substitution of NaCl by KCl. Briefly, the first technology consisting in pre-drying meat appeared relevant to reach a maximum 26% sodium reduction. However, two hazards have to be taken into account when applying this technology, namely (1) the duration of the meat pre-drying step, which is to be limited to a maximum of four days in order to avoid *Pseudomonas* growth, and (2) the final a_w of the DFS at the end of drying, which has to be lower than 0.92 and thus requires sufficient DFS weight losses, especially if producing leaner sausages. The second technology corresponding to a pre-drying of the sausages before the fermentation stage also appeared relevant to achieve a maximum 24% sodium reduction. However, the DFS treated using this pre-drying technology presented higher a_w values (0.91) at the end of drying compared to control DFS (0.88), which could prove damaging in terms of shelf-life. Finally, the third technology, i.e. partial NaCl substitution by KCl, appeared to be a good way forward and easier to set up in practice than the first two technologies to reduce final sodium content in DFS. Applying 30% substitution (w/w) of

NaCl by KCl had no significant impact on weight losses, pH kinetics or sensory attributes of DFS and led to final a_w values close to control DFS.

To reduce SFA content by 60%, two alternative technologies were investigated in Task 2.1 and reported in Deliverable D2.1: (i) direct suppression of animal fat and (ii) partial substitution of animal fat by cryo-crystallized vegetable fat, such as oleic sunflower oil which is rich in mono-unsaturated fatty acids. Finally, technological problems (significant oil loss, emergence of large cracks and holes and obvious structural defects) meant that incorporating cryo-crystallized vegetable fat in meat batter or using vegetable fat emulsion in sausages to lower SFA content appeared inappropriate as technological solutions for DFS manufacture. The only strategy that can be reasonably set up in practice to reduce SFA content by 60% consists in directly reducing pork fat addition in DFS through the use of lean pork meat containing only 7% fat.

2.2 Approach engaged in this research

With the focus on DFS, the main objectives of WP2 are to develop procedures allowing the production of safe, nutritionally improved products that are acceptable to consumers. The binary reduction goal targeted for DFS is a 30% reduction in sodium and a 60% reduction in SFA. In this context, the planned objective of Task 2.3 is to investigate, at lab scale, the impact of reducing animal fat and salt contents on water and salt transfers and the formation of odour and flavour compounds. Indeed, removing sodium and fat from DFS will alter the inner biochemical reactions (proteolysis, fermentation, oxidations...) due to changing water activity (a_w), and thus the final product aroma, flavour and taste.

On account of the main findings reported in Deliverable 2.1 and in accordance with the main results of the literature, the concrete objective set for Task 2.3 was to run two series of DFS manufacture to bring accurate data on the following three points:

- The potential application of new technologies in DFS production (adding KCl as a substitute for NaCl, adding vegetable oil...) to lower SFA and sodium content,
- The impact of fat and salt reduction on water and salt transfers and the formation of odour and flavour compounds,
- The product quality and consumer acceptability of low-sodium and low-fat DFS.

It was decided early on to uncouple the experimental study of water transfers from the study of aroma production and to complete these studies by a time–course study (on Day 1, D7, D21, and D29) to track and trend key biochemical parameters such as pH, proteolysis, fermentation and protein and lipid oxidations during the fabrication of DFS containing different amounts of sodium and animal fat. These biochemical phenomena are responsible for the production of aroma within the DFS. Since these biochemical analyses are fully destructive and need several repetitions, large numbers of DFS had to be fabricated and dried for about one month. For practical reasons, we thus used the pilot ripening rooms of our partner ADIV to manufacture these DFS instead of our laboratory's purpose-built micro-bioreactors (MBRA) in which only one DFS can be manufactured at a time. In total, two series of DFS were manufactured, representing 15 different batches of about 30 units per batch. The first series was designed to investigate the effect of reducing NaCl and animal fat content on the time–course of physicochemical parameters such as pH, weight loss and a_w and biochemical parameters such as proteolysis, lipolysis, fermentation, protein oxidation and and lipid oxidation. For the 8 batches corresponding to this first series, the animal fat and sodium content of each batch was fixed by building a Doehlert experimental design with two factors. In parallel, trials were carried out in which the ultimate drying stage was performed in our lab MBRAs. In the second series, the 7 batches for manufacture were chosen based on input from Task 2.1 on the beneficial use of new strategies (adding

KCl and/or vegetable oil) to reduce sodium and SFA content as reported in deliverable D2.1 and on the preliminary results highlighting that flavouring played a crucial role in French consumer perceptions of the typical DFS aroma. For all DFS batches, quality was assessed by texture profile analysis (TPA) tests, and in the specific case of the second series manufactured, by sensory descriptive analysis and consumer acceptability testing by a panel of 29 assessors accustomed to eating DFS.

In addition, while waiting for all the DFS to be manufactured, we led a special study to better understand the biochemistry of the aroma of DFS by identifying the odour-active molecules and their respective biochemical origins (from meat or fat, from flavouring, from unknown origins) using gas chromatography–olfactometry. These analyses were performed on products purchased commercially from producers identified by the laboratory in previous projects as manufacturing typical products presenting a sharp aroma of DFS. The same type of aroma analysis was also performed on DFS manufactured in the second series of fabrication, with the particular—but challenging—objective of finding interrelationships between aroma production, biochemical evolution and sensory acceptability by consumers.

3. Materials and Methods

3.1 DFS manufacture

As previously indicated (section 2.2), two series of 15 batches of DFS were manufactured.

3.1.1 First series of DFS fabrication

The first series of 8 batches was designed to investigate the effect of reducing NaCl and animal fat content on the time-course of key physicochemical parameters (pH, weight loss and a_w) and biochemical parameters (proteolysis index, acid lactic, protein oxidation based on evaluation of carbonyl and free thiol groups contents and lipid oxidations based on quantification of TBARS and HSB values). The animal fat and NaCl content of each batch was fixed by building a Doehlert design (Doehlert, 1970) with two factors: initial salt content in the range [2.0%-2.8%] and initial animal fat content in the range [8.4%-21%]. It is important to note that in a Doehlert design, the number of levels is not the same for all variables: in a two-factor problem, the first factor (i.e. salt content) is studied at three levels (2.0%, 2.4% and 2.8%) and the second factor (animal fat content) at five levels (8.4%, 11.6%, 14.7%, 17.9% and 21.0%). This particular design enabled us to reduce the number of fabrications needed to 7, with an eighth 'control' fabrication in which DFS were manufactured from a 2.8% initial salt content and a 21% initial fat content used as baseline reference for the other fabrications. Table 1 details all the batches of the first series of fabrication.

Table 1. Details of all the batches of dry-fermented sausages made during the first series of fabrication. The animal fat and NaCl content of each batch was fixed by a Doehlert design with two factors and expressed as % of total matter (TM).

Experiment	Animal fat content (% TM)	NaCl content (% TM)
Experiment 1	14.7	2.4
Experiment 2	21.0	2.4
Experiment 3	17.9	2.8
Experiment 4	8.4	2.4
Experiment 5	11.6	2.0
Experiment 6	17.9	2.0
Experiment 7	11.6	2.8
Experiment 8 (Control)	21.0	2.8

For each formulation in Table 1, about 30 DFS were manufactured by a technician at the ADIV platform according to the following procedure. Raw pork meat (shoulder and pork backfat) was purchased from a local distributor (DISTRIPORC, Clermont-Ferrand, France) and the pH and a_w values of the lean pork meat were verified at the beginning (pH = 5.98 and a_w = 0.97). Pork shoulders were defatted and cut into small parallelepipeds. For each formulation, the corresponding amount of defatted pork shoulder and backfat was weighed, ground to 6 mm diameter, and mixed with a set of additives and a starter culture corresponding to a mid-acidification kinetic starter. The starter culture was prepared at 100 kg/L concentration and added to each formulation at 10 g/kg. In each meat batter, we added dextrose (5 g/kg), potassium nitrate (0.3 g/kg), potassium erythorbate (0.5 g/kg),

black pepper (2 g/kg), garlic powder (0.5 g/kg) and, finally, a solution of starters (10 g/kg). The meat batter was then stuffed into 50 mm-diameter collagen casings. The raw sausages, weighing 450 g and about 20 cm in length, were then plunged into a *Penicillium nalgiovensis* solution to cover the DFS surface during the drying stage. All the products were steamed for 4 days at 24°C and 70% relative humidity (RH), then dried for 25 days at 13°C and 70% RH in the same ripening room. For the 8 batches of Table 1, one sample at Day 0 (meat batter before stuffing) and 3 sausages at Day 1, 2, 5, 7, 14, 21 and 29 of drying were taken to evaluate the time-course patterns of chemical composition, physicochemical parameters (a_w, weight loss, pH) and biochemical parameters (proteolysis, fermentation, protein oxidation, lipid oxidation). Given how experiments were cumbersome to set up, the biochemical parameters were finally only assessed at five timepoints, i.e. Day 0, 1, 7, 21 and 29. In addition, for biochemical and basic chemical analysis, all the DFS were individually treated with liquid nitrogen and ground down into fine powder to minimize problems tied to heterogeneity of sampling in subsequent analysis, and the powder was stored at -80°C until analysis.

3.1.2 Second series of DFS fabrication

The composition of the 7 batches of the second series of fabrication (Table 2) was defined based on Task 2.1 outcome highlighting the benefits of incorporating KCl and vegetable oil to reduce sodium and SFA content and on early Task 2.3 findings indicating that flavouring (especially garlic) played a crucial role in French consumer perceptions of the typical DFS aroma (see 4.3.1). Table 2 details all the batches of this second series of fabrication.

Table 2. Details of all the batches of dry-fermented sausages made during the second series of fabrication. The animal fat, sunflower oil, NaCl and KCl content of each batch is expressed as % of total matter (TM). Garlic powder content is expressed in g per kg.

Experiment	Animal fat content (% TM)	Sunflower oil content (% MT)	NaCl content (% TM)	KCl content (%TM)	Garlic powder content (g/kg)
Experiment 9	21.0	0	2.8	0	0
Experiment 10 (Control)	21.0	0	2.8	0	0.5
Experiment 11	21.0	0	2.0	0.8	0.5
Experiment 12	7.0	0	2.8	0	0.5
Experiment 13	7.0	3.0	2.8	0	0.5
Experiment 14	7.0	0	2.0	0.8	0.5
Experiment 15	7.0	3.0	2.0	0.8	0.5

As for the first series of DFS fabrication, about 30 DFS were manufactured for each batch of Table 2 by an ADIV technician. Series 2 was manufactured following the same procedure as series 1 and with the same amounts of additives, i.e. dextrose at 5 g/kg, potassium nitrate at 0.3 g/kg, potassium erythorbate at 0.5 g/kg, black pepper at 2 g/kg, garlic powder at 0.5 g/kg (except for experiment 9), and a solution of starters at 10 g/kg. The only difference in DFS manufacture lies in the fact that the incorporation of 3% TM oleic sunflower oil (previously stored at -2°C) in the DFS of experiments 13 and 15 (Table 2) required the prior preparation of an emulsion with lean pork meat and the addition of 1% TM wheat plant fibres (WF200, Rettenmaier & Son, Rosenberg, Germany) to bind the

batter and minimize further oil loss in liquid form. Like series 1, once stuffed, the raw sausages were also soaked in a *Penicillium nalgiovensis* solution, fermented at 24°C and 70% RH for 4 days and dried at 13°C and 70% RH for about 25 days. For the 7 batches of Table 2, similarly to the first series of fabrication, one sample was taken at Day 0 (meat batter before stuffing) and 3 sausages were taken at Day 1, 2, 6, 8, 14, 21 and 29 of drying to evaluate the time-course patterns of chemical composition, physicochemical parameters and biochemical parameters. As for experiments 1 to 8, the biochemical parameters were assessed only at five timepoints: Day 0, 1, 8, 21 and 29. All the DFS were individually treated with liquid nitrogen and ground down into fine powder to minimize problems tied to heterogeneity of sampling in subsequent basic chemical and biochemical analysis, and the powder was stored at -80°C until analysis.

3.2 MBRA drying trials

In the INRA-QuaPA unit, an experimental device was set up using air-product water balance to non-destructively estimate the time-course of mean a_w at the food product surface under well-controlled airflow conditions (Le Page et al., 2010). The so-called 'MBRA' device is especially suited to studying the ripening of cheeses and fermented meat products, where water fluxes exchanged between products and air are very low. The validation tests performed with a_w-known model products showed that water fluxes of 10^{-7} $kg.s^1$ can be estimated with an accuracy better than 2% over very short periods of time (10 min), and that surface a_w can be estimated with an absolute uncertainty of less than 0.01 a_w units. Figure 1 gives a schematic description of the MBRA built, including the 7 measurement points distributed along the MBRA air circuit (6 points) and over the product surface (1 point). Care was taken to control the air characteristics inside the ripening cell. Air temperature around the food product is easily controlled in the range 6-20°C via the air temperature of the coldroom in which the MBRA is placed. Mean air velocity around the food product can be set in the range 2-12 $cm.s^{-1}$ via a fan located after the ripening cell, and whose airflow rate can be adjusted by modifying the electrical supply in the range 5-12 V. Regarding the RH of air around the product, high values up to 99% can be obtained and controlled by recycling a part of the humid air leaving the cell by means of an automated 3-way valve (Figure 1).

Briefly, working from air-product water balance across the MBRA ripening cell, mean water flux evaporated from the cheese surface (annotated q_{evap}) can be simply expressed as:

$$q_{evap} = dm2 \,.\left(1 - \frac{dm3}{dm2}\right).(X_{recycling} - X_{inlet}) \quad (1)$$

where dm2 and dm3 are the dry mass airflow rates flowing around the product and in the recycling duct, respectively, and $X_{recycling}$ and X_{inlet} are the water contents in the air flowing in the recycling duct and at the MBRA inlet, respectively, i.e. before and after the food product placed in the drying/ripening cell. In addition to q_{evap}, mean $a_{w,surf}$ can be estimated from the equation of convective drying of wetted solids, from:

$$a_{w,surf} = \left[\left(\frac{q_{evap}}{k \,.\, S_{prod}}\right) + P_{air}\right] / P_{sat(T_{surf})} \quad (2)$$

where k is the mass transfer coefficient ($kg.Pa^{-1}.m^{-2}.s^{-1}$), which is determined as a function of air velocity by psychrometry from independent experiments; S_{prod} is total product surface (m^2); P_{air} is partial water vapour pressure in the air (Pa) around the product,

derived from ripening-cell air temperature and water content; and $P_{sat(Tsurf)}$ is saturated water vapour pressure (Pa) at product surface temperature.

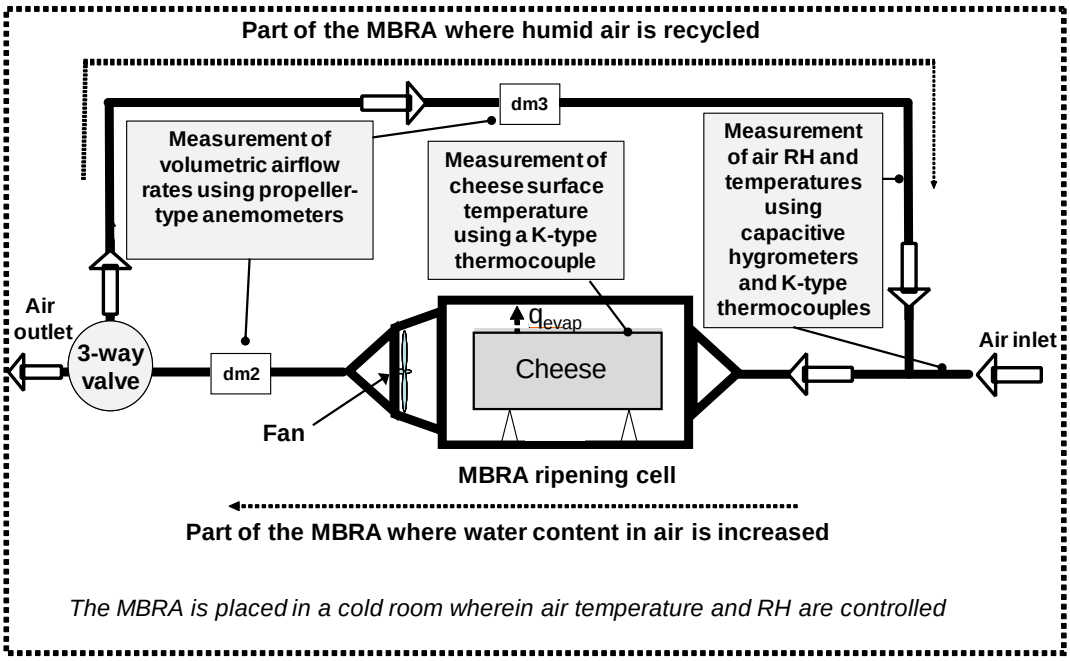


Figure 1. Schematic view of the micro-bioreactor (MBRA) built in the INRA-QuaPA unit specifically to study air-product surface water exchanges in order to estimate the time-course patterns of evaporated water fluxes and mean surface a_w.

Applied to cheese ripening, the MBRA trials highlighted (1) effects of low air velocity and high RH on the water exchanges occurring at the cheese surface, demonstrating strong surface sensitivity to external ripening air conditions, and (2) a close interaction between surface microbial flora development and the water transfers occurring both from cheese core to surface and at the surface itself (Le Page et al., 2012).

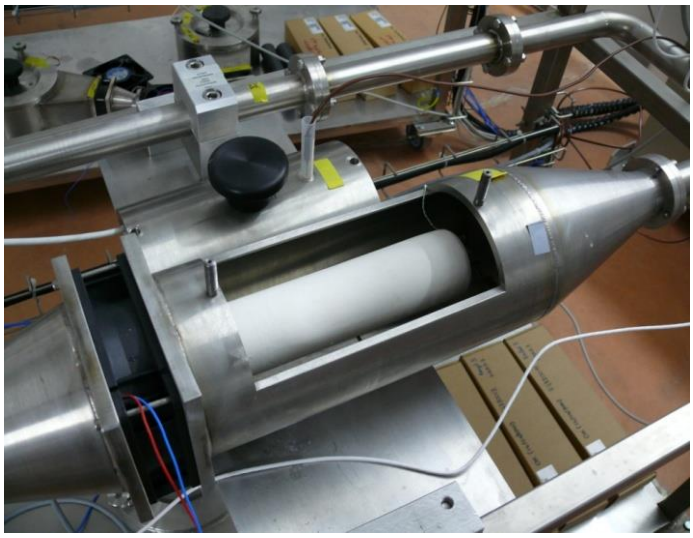


Figure 2. View of a new drying cell specifically installed in the micro-bioreactors (MBRA) to study air-raw sausage surface water exchanges.

Under the TeRiFiQ project framework, new cylindrical drying/ripening cells were built and implemented in the MBRAs (Figure 2) in place of the circular cells initially installed which were not really suitable for DFS drying. Moreover, MBRA operation was adapted to enable these lab devices to perfectly reproduce what happens in industrial ripening rooms in terms of air velocity and RH (drying) conditions applied around the DFS.

In addition to the study of air–DFS surface water exchanges, in an effort to indirectly quantify biological activity inside the DFS and microbial flora activity at the DFS surface, we designed and built a plastic box to capture and measure CO_2 production. Its size was chosen to keep air volume surrounding the DFS low in order to obtain *a priori* a rapid evolution in CO_2 content inside the box. During experiments, the product has to be removed from the MBRA and transferred into the box, every two days, in order to evaluate CO_2 production rate. The two experimental devices, i.e. MBRA and plastic box, were placed in the same coldroom so that measurements were not influenced by change in air temperature. The DFS is placed inside the purpose-built box for 120 minutes to enable CO_2 content to reach a measurable value. Three air samples are then taken via a needle connected to a commercially-available gas analyzer (Gaspace III, Gruter & Marchand, Nanterre, France). Each air sample lasted 8 s and corresponded to a gas flow rate of 250 $cm^3.min^{-1}$, with an uncertainty of ±12.5 $cm^3.min^{-1}$. Before taking each air sample, the air was mixed for 10 min using a magnetic agitator to homogenize in-box CO_2 content.

3.3 Chemical, physicochemical and biochemical analysis

3.3.1 DFS chemical composition

3.3.1.1 Water content

In-DFS water content was determined by drying about 1.5 g of powdered-down sample at 80°C ± 2°C in a controlled-temperature chamber (Model FT127U, Firlabo, France) to constant weight, i.e. at least 48 h. Moisture content was expressed on a total matter (TM) basis (kg H_2O/kg TM). All water content measurements were performed in 6 replicates.

3.3.1.2 Salt content

In-DFS salt content was measured using 2 g of powdered-down sample. The sample was homogenized (Ultra-Turrax system, Ika, Germany) with 20 mL of ultrapure water. After a 3-hour rest period, the homogenate was centrifuged at 11,300 *g* for 10 min at room temperature (MiniSpin Plus, Eppendorf, France). The supernatant was recovered, diluted in ultrapure water, and run through an ion chromatography system (850 professional IC, Metrohm France SAS, France) to systematically measure chloride ion and sodium ion contents. The chloride ion or sodium ion values were then used to calculate an equivalent NaCl content (%). KCl content was also quantified for DFS in which NaCl had been partially substituted with KCl to evaluate current KCl content. All salt content measurements were performed in 6 replicates.

3.3.1.3 Fat content

In-DFS fat content was determined on powdered-down samples based on the method of Folch et al. (1957) but using dichloromethane/ethanol (2:1) instead of chloroform/methanol (2:1) as solvent. Total lipids from 0.5 g of sample were extracted with 50 mL of solvent. The organic phase (dichloromethane) containing total lipids was separated using 10 mL of salt solution at 0.73%. The extract obtained was evaporated in a vacuum evaporator and weighed to determine total lipid content. All measurements were performed in 6 replicates.

3.3.2 Physicochemical analysis

3.3.2.1 Weight loss

Throughout the DFS drying period, 9 products from each batch arranged on the same bar were weighed together practically every day to determine the kinetics of weight loss. Weight loss was expressed as percentage of the initial weight.

3.3.2.2 a_w

In-DFS water activity (a_w) was measured at 20°C with an a_w-meter (a_w Sprint TH-500, Novasina, Switzerland). Preliminary tests performed to measure a_w individually on the three sausages of each formulation showed that there was no significant difference between the three values (<0.001 a_w unit). a_w values were therefore determined on a mixture of the three batches for each formulation of the two series of fabrication.

3.3.2.3 pH

In-DFS pH was determined using 1 g of powdered-down DFS sample homogenized (Ultra-Turrax system, Ika, Germany) with 10 mL of ultrapure water. pH was measured conventionally with a pH meter (InLab427, Mettler Toledo, France) calibrated with standard solutions of pH 4 and pH 7. All pH measurements were performed in 9 replicates.

3.3.3 Biochemical analysis

3.3.3.1 Proteolysis index

The proteolysis index (PI) of each powdered-down DFS sample was determined to evaluate intensity of proteolysis using the fluorescamine-based method detailed in Harkouss et al. (2012). In this method, PI is defined as the percentage of the ratio of amino acids and peptides (N-terminal α-amino group) content to total protein content. All PI measurements were performed in 9 replicates.

3.3.3.2 Lipolysis

The degradation of fat into fatty acids (lipolysis) was quantified by determining the acid value of fat (norm NF T 60-204) in DFS samples at Day 29. Briefly, total free fatty acids from 25 g of samples were solubilized in a solvent mix of ether/ethanol. Total free fatty acids were determined quantitatively by potassium hydroxide (0.1 N) in the presence of a colour indicator (phenolphthalein). Before neutralization, phenolphthalein (acid medium) is colourless. Under basic conditions (beyond neutralization), it is coloured in pink. The number of equivalents of potassium hydroxide poured is equal to the number of equivalents of acid present in the sample, and acid value is the mass of potassium hydroxide, in mg, required to neutralize one gram of fat.

3.3.3.3 Lactic acid content

In-DFS lactic acid content was measured in powdered-down samples via the following method. One g of sample was homogenized in 0.5 M perchloric acid. After centrifugation for 20 min at 2500 *g*, the supernatant was recovered for lactic acid determination. All lactic acid content measurements were performed in 9 replicates.

3.3.3.4 Lipid oxidation

In-DFS lipid oxidation was quantified in powdered-down samples via two complementary experimental methods: determination of (i) thiobarbituric acid reactive substances (TBARS) and (ii) hydrosoluble Schiff bases (HSB).

TBARS were determined using the method developed by Mercier et al. (1998) based on the technique of Lynch & Frey (1993). Muscle samples (1 g) were homogenized with 10 mL of deionized distilled water using a Polytron blender (1 min at medium speed). Homogenates (0.5 mL) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 mL) and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath for 10 min. After cooling at room temperature for 30 min, the pink chromogen was extracted with n-butanol (2 mL), and its absorbance measured at 535 nm against a blank of n-butanol. TBARS concentrations were calculated using 1,1,3,3-tetraethoxypropane (0–0.8 µM) as standard. Results are expressed as mg of MDA per kg of meat (TBA units).

Lipid oxidation in DFS samples was also determined by quantifying HSB. To do this, the aqueous phase obtained to determine fat content was collected to quantify HSB levels. In parallel, a standard curve of commercial Schiff bases (quinine) was prepared. Fluorescences of each point of the standard curve and each sample were measured with a spectrofluorometer (FP 8300, Jasco France, France) under a 370 nm excitation wavelength (excitation slit of 10 nm), a 470 nm emission wavelength (emission slit of 10 nm) and an integration time of 3 s. A linear standard curve of quinine was plotted, and sample HSB levels of were expressed as micromoles/kg meat *vs*. quinine equivalent. All lipid oxidation measurements were performed in 9 replicates.

3.3.3.5 Protein oxidation

In-DFS protein oxidation was assessed in powdered-down samples via two experimental methods consisting in quantifying (i) carbonyl groups and (ii) free thiol groups of cysteine residues.

The carbonyl groups were quantified using the method of Mercier et al. (1998) adapted to meat based on the technique developed by Oliver et al. (1987). This technique detects carbonyl groups based on the 2,4-dinitrophenylhydrazone formed after reaction with 2,4-dinitrophenylhydrazine (DNPH) on the carbonyl groups. The adduct is assayed by spectrophotometry at 370 nm. The results are expressed in nanomoles of bound DNPH per mg of proteins.

The free thiol groups of cysteine residues were determined based on Ellman's reaction. The Ellman assay was run according to the protocol of Morzel et al. (2006) that uses 2,2′-dithiobis(5-nitropyridine) (DTNP) as reagent. In alkaline solution, DTNP binds to the anionic free thiol groups of cysteine residues to form a complex that absorbs at 386 nm. The results are expressed in nanomoles of bound DTNP per mg of proteins. All protein oxidation measurements were performed in 9 replicates.

3.4 DFS aroma analysis

3.4.1 Identification of odour-active compounds in high-quality DFS

In this section, the objective was to identify the odour-active compounds responsible for the characteristic aroma of DFS in order to find the simplest way to enhance the aroma of new low-fat low-salt products. To this end, we implemented powerful methods of identification using high-resolution gas-phase chromatography and mass spectrometry

coupled with single- or multi-way olfactometry. The structural identification and odour characteristics of the key compounds in the aroma will enable us to trace their most likely origins (meat biochemistry, flavouring, etc.).

3.4.1.1 DFS sample origins

The odour-active compounds were identified on commercially available DFS. The dry sausages, selected for their intense aroma, were sourced from:

- "Salaisons de Fix", Le Bourg, 43320 Fix-Saint-Geneys, France (http://www.salaisondefix.fr).
- "Salaisons du Lignon": 160 rue Chazelet BP 12, 43200 Saint-Maurice-de-Lignon, France (http://www.salaisons-du-lignon.com).
- "Salaisons Debroas" Le Bas-de-Celas, 07110 Largentière, France (http://www.ardeche-agroalimentaire.fr/entreprise/SALAISONS-DEBROAS_103).
- "Salaisons Pyrénéennes" 2 rue Anatole-France, 65320 Bordères-sur-l'Echez, France (http://www.salaisons-pyreneennes.com/).
- "SARL Bornes" 4 place de la Mairie, 15400 Trizac, France. (http://www.boucherie-bornes.fr/).

3.4.1.2 Solid-Phase Microextraction–Comprehensive Gas Chromatography–Time-of-Flight Mass Spectrometry (SPME–GCxGC–tofMS)

Five grams of minced DFS were placed at ambient temperature in a sealed 20 mL vial and pre-incubated for 10 min. The extraction of volatiles was performed using a 75 mm Solid-Phase MicroExtraction (SPME) Carboxen/PDMS fiber (Supelco Bellefonte, PA). The fiber was exposed to the DFS sample headspace for 1 h at ambient temperature. After extraction, a splitless injection (at 220°C for 2 min) of volatiles was carried out with an SPME Combipal autosampler (CTC Analytics AG, Zwingen, Switzerland). Separation and detection was performed using a LECO Pegasus IV GCxGC-MS-tof system (LECO Corporation, St. Joseph, MI). Volatiles were separated first on a RTX-5 capillary column (length 30 m, internal diameter 0.32 mm, film thickness 1 µm; Supelco, Saint-Germain-en-Laye, France) and second on a DB-17 capillary column (length 2.50 m, internal diameter 0.178 mm, film thickness 0.30 µm; J&W Agilent, Santa Clara, CA). Volatiles were detected by electronic ionization at 70 eV, and ions were acquired over the range 18 < m/z < 220 a.m.u. at 200 scan.s^{-1}. Further details on the analytical settings can be found in Théron et al. (2010).

3.4.1.3 Dynamic Headspace Sampling–Gas Chromatography–Mass Spectrometry (DHS-GC-MS).

Ten grams of DFS were minced and placed at ambient temperature in a Pyrex® extraction cartridge (ref. M3, Maillères, Aubière, France). Volatiles were then extracted by DHS (Tekmar, Cincinnati, OH 45234, USA) for 60 min with a helium stream (Messer, He/U purity: 99.995%) at a flow rate of 40 mL.min^{-1}. The trap (Tenax® TA 60/80 mesh adsorbent, Supelco Bellefonte, PA; working length 180 mm and inside diameter 1/8") was operated at 30°C. The dry purge step was set at 30 min. The volatile components were then desorbed from the trap at 180°C for 10 min using helium (He/N55 purity: 99.9995%) and sent into the cryo-focalization area (cooled at −150°C with liquid nitrogen). After injection, the trap was heated at 180°C for 2 min to separate and identify the volatiles using a GC–MS setup composed of a chromatograph (GC 6890, Agilent Technologies; apolar capillary column RTX5-MS, length 60 m, internal diameter 0.32 mm, film thickness 1 µm) linked to a 5973 Inert MSD-series quadrupole mass analyzer (Agilent Technologies).

Volatiles were detected by electronic ionization at 70 eV, and ions were scanned over the range 18 < m/z < 220 a.m.u. For the trials with precursors, the sulphur-based odourants-of-interest were semi-quantified by measuring their peak areas from specific ions acquired in single-ion monitoring mode.

3.4.1.4 Gas Chromatography–Mass Spectrometry/Olfactometry

Two complementary olfactometry instruments were used to detect and identify odour-active compounds. Both were coupled to identical dynamic headspace devices (Tekmar, Cincinnati, OH 45234, USA) and the volatile extraction conditions were similar to the conditions described in section 3.4.1.3.

The first instrument (Figure 3) made an exhaustive inventory of odour-active compounds by eight-way olfactometry coupled to mass spectrometry (DHS-GC-MS/8O) (Berdagué & Tournayre, 2005; Berdagué et al., 2007).

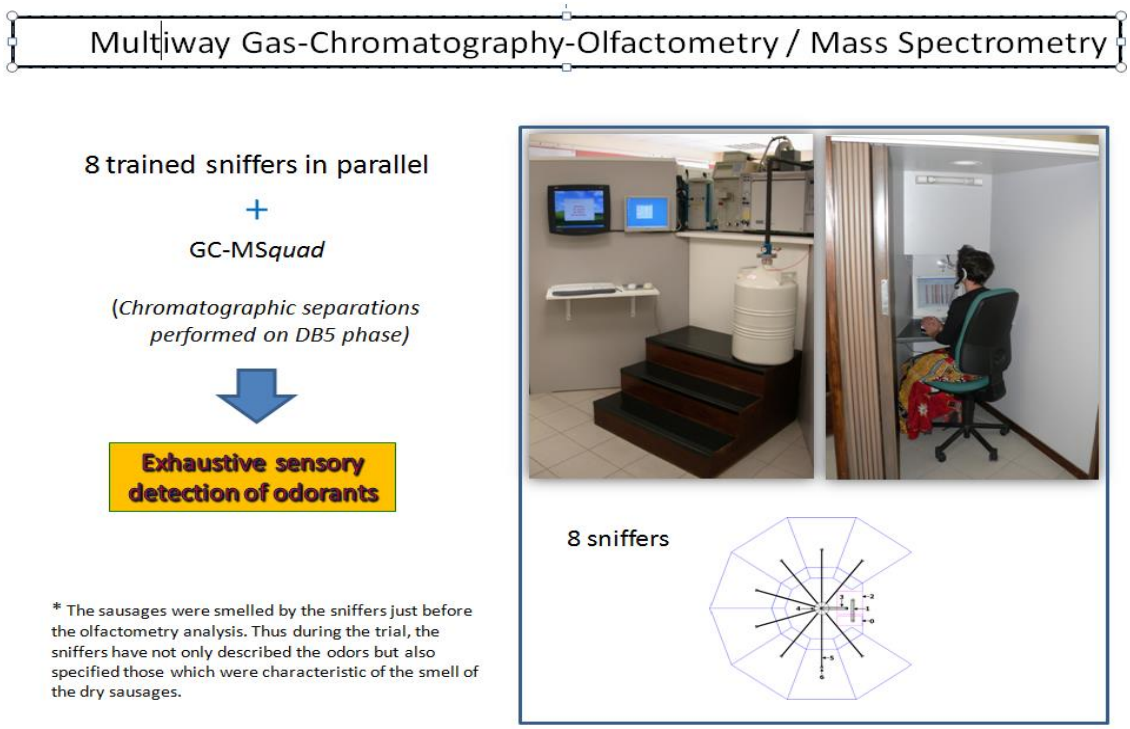


Figure 3. View and schematic description of the Multiway olfactometry device coupled to Gas-Chromatography–Olfactometry and Mass Spectrometry.

This system is able to detect a very large number of olfactory zones by accumulating the performances of eight sniffers. The selected sniffers were non-smokers with no known health disorders, aged under 40, and with attested sensitivity and ability to detect and consistently describe a wide range of odours. The nature of the samples analyzed was communicated to the sniffers, who were asked to focus on the "meat, dry-fermented sausage" odour. To measure the intensity of the odours, a five-level scale (1, very weak; 2, weak; 3, moderate; 4, strong, and 5, very strong) was used. The olfactometric analyses lasted 40 min. Olfactometric data were acquired and processed using AcquiSniff® Software (Berdagué & Tournayre, 2002; Tournayre & Berdagué, 2003). Two sniffing sessions with eight sniffers per session were run on the DFS samples with the DHS-GC-MS/8O device. The aromagram of the DFS samples was obtained from the mean intensities of the individual aromagrams (Berdagué & Tournayre, 2002).

The second instrument (Figure 4) was a gas chromatography-mass spectrometry-olfactometry system with a single olfaction port.

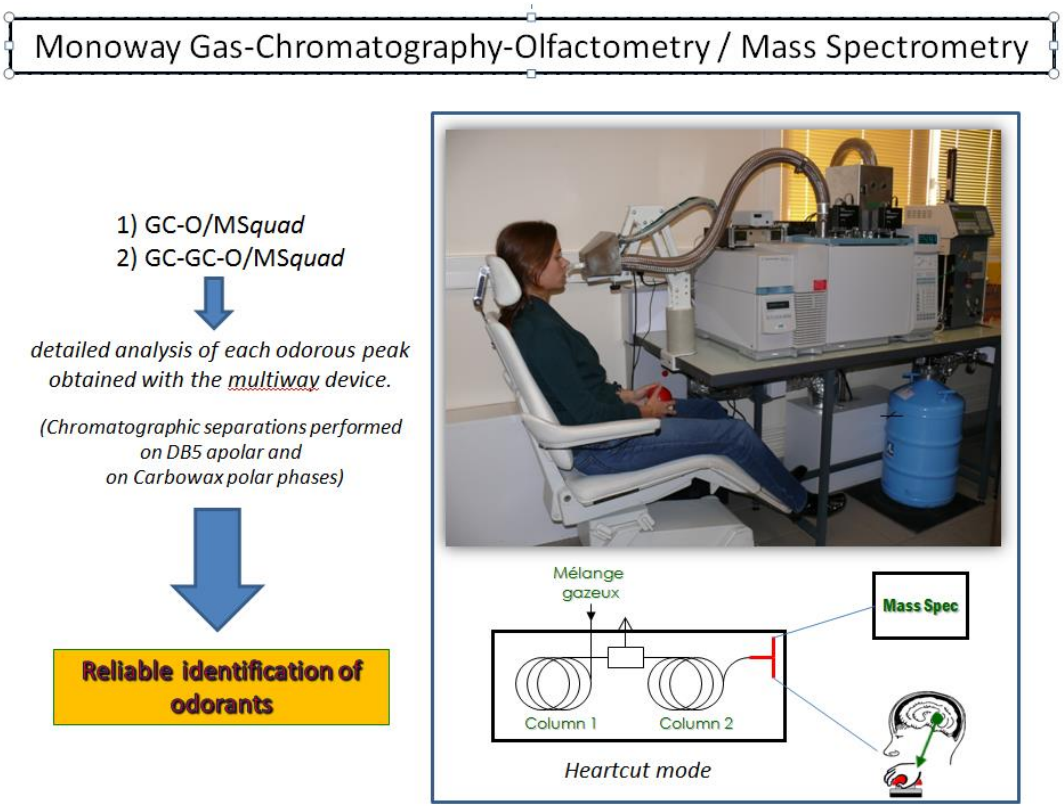


Figure 4. View and schematic description of the two-dimensional "heart-cut"-type Gas-Chromatography-Olfactometry coupled to mass spectrometry system used in the laboratory.

This instrument worked either in GC-MS-olfactometry mode (DHS-GC-MS/O) to fit DHS-GC-MS/8O analysis or in two-dimensional gas chromatography mode (DHS-GC-GC-MS/O, also termed "heart-cut mode") to perform a detailed olfactory exploration of all the odour-active zones observed by DHS-GC-MS/8O, which is essential for reliable identification. The DHS-GC-MS/O or DHS-GC-GC-MS/O instrument was composed of a chromatograph (GC 6890, Agilent Technologies; capillary column RTX5-MS, length 60 m, internal diameter 0.32 mm, film thickness 1 µm) hyphenated to a quadrupole mass detector (MSD 5973 Inert, Agilent Technologies). The capillary column was connected to the mass spectrometer via a deactivated capillary column (SGE, length 0.5 m, internal diameter 0.10 mm) and to the sniffing port via a deactivated capillary column (SGE, length 1.7 m, internal diameter 0.32 mm) using a zero-dead-volume T-connector (inside tubing diameter 1/16", ZTIM Valco® Instruments, Houston, TX). The ratio of effluent between sniffing port and mass detector was 1/1. For the DHS-GC-GC-MS/O mode, the volatile compounds from the first separation on the apolar column (RTX5-MS) were cryofocusses on a second polar column (DB-WAXETR, J&W Scientific, Agilent Technologies: length 30 m, internal diameter 0.32 mm, film thickness 1 µm), and then sent by heating (180°C, 2 min) to the sniffing port and to the mass spectrometer. Olfactometry data obtained by DH-GC-MS/O or by DHS-GC-GC-MS/O were both acquired by two assessors using the AcquiSniff® Software under similar conditions (vocabulary, odour intensity (1-5) and odour duration) to those used for DHS-GC-MS/8O data. The odour of the candidate structures identified by mass spectrometry was compared with their odour as described in several databases (http://www.thegoodscentscompany.com, http://www.flavornet.org), and with their odour after co-injection of pure reference compounds on apolar and polar phases.

3.4.2 Analysis of the volatile fraction of low-fat low-salt flavoured DFS

The objective of this section was to study the effect of different DFS formulations on several markers of the main chemical and biochemical pathways involved in aroma formation, namely degradation of lipids and amino acids and catabolism of carbohydrates. To this end, a series of markers were selected. Markers of flavouring were also monitored. These markers were analyzed by DHS/GC–MS.

In concrete terms, the volatile compounds were extracted and analyzed using the method described in section 3.4.1.3. The volatile markers monitored in the study concerned the catabolism of carbohydrates (2,3-butanedione), oxidation of lipids (hexanal, heptanal, octanal, nonanal, 2-heptanone, 2-octanone, 2-nonanone, 1-octen-3-ol, 1-octen-3-one), catabolism of amino acids (butanal, 3-methylbutanal, 2-methylbutanoic acid, 3-methylbutanoic acid, 2-methylethanol, 2-phenylethanol) and flavouring (2-propene-1-thiol, 1-propene, 3-methylthio or sulphide, allyl methyl, disulfide, methyl allyl, alpha-pinene).

3.5 Evaluation of DFS quality and sensory acceptance

3.5.1 Texture profile analysis

DFS quality was first evaluated objectively through texture measurements performed on non-frozen 30×20×50 mm parallelepiped samples extracted from 29-day-old DFS. In collaboration with ADIV, a TA.XT Plus universal texture analyzer (Stable MicroSystems Ltd., Surrey, England) was used to perform the texture profile analysis (TPA) test (Bourne, 2002) at room temperature (Figure 5). The 20 mm-high parallelepiped samples were placed under the compression plate surface (flat 490 mm^2 cross-section plunger) and compressed axially twice to 50% of their original height with a time interval of 2 s between the two successive compressions. Force–time curves similar to the example represented in Figure 6 were recorded with a 15 kg load cell applied at a crosshead speed of 1 mm/s.

Figure 5. View of the texture profile analysis equipment used in this study to investigate the texture of all the DFS manufactured.

The following TPA parameters were obtained using the XT.RA Dimension Specific software package delivered with the experimental device: hardness, fragility, elasticity, adhesiveness, and cohesiveness. Hardness was defined by peak force of the first compression cycle and expressed in N, and fragility by the ratio of the peak force in the second compression cycle to peak force of the first compression cycle. Cohesiveness was calculated as the ratio of area under the second curve to area under the first curve. Elasticity was defined as the ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Adhesiveness corresponded to the "negative" area under the curve obtained between the two cycles, expressed in N.s.

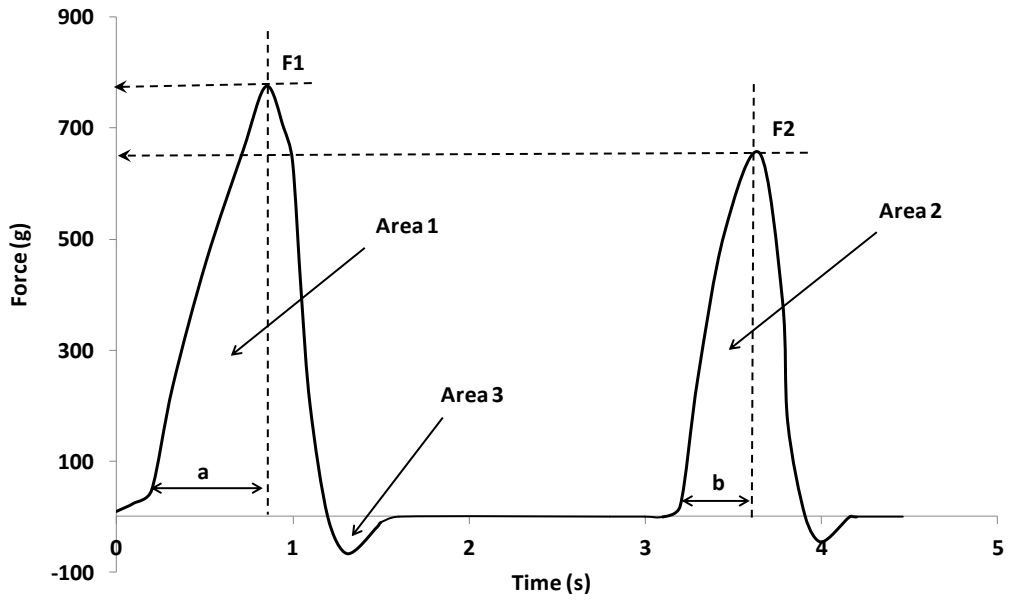


Figure 6. Example of a typical result obtained after a texture profile analysis (TPA) test performed on a meat product sample.

The mean ± standard deviation (SD) of the 6 samples of each DFS batch was used for statistical analyses. An analysis of variance (ANOVA) was performed to assess the effect of salt and animal fat content on all texture measurements. When ANOVA found a significant effect, a post-hoc procedure (Tukey test) was used.

3.5.2 Sensory acceptability of low-fat and low-salt formulations

DFS quality was also evaluated through a sensory analysis performed by 29 assessors with the objective of comparing consumer-panel acceptability of flavoured and non-flavoured low-salt and low-fat products.

3.5.2.1 Scoring

Scoring was carried out by the 29 assessors using a structured scoring scale (0–10). Eight samples were presented to the assessors. The first sample corresponded to the formulation "NaCl 2.8% + fat 21% + flavouring", i.e. the formulation of the control DFS, and was designed only to habituate the assessors to the tasting task. The other 7 formulations corresponded to the 7 batches of the second fabrication series (experiments 9-15) and were presented to the judges in random order, and only their sensory evaluation results

were analysed statistically. Four DFS acceptability criteria were studied—appearance, texture, taste and aroma.

3.5.2.2 Statistical analysis

Hierarchical cluster analysis (HCA) was used to typologize the assessors according to how acceptable they found the 7 formulations tested based on the DFS acceptability variables appearance, texture, taste and aroma. The calculations were carried out according to the method of Ward (STATISTICA 10-V2014). Effect of formulations on acceptability scores for product appearance, texture, taste and aroma was evaluated by ANOVA using the models:

$$Acceptability\ score_{i,j,k} = \mu + Assessor_i + Formulation_j + Assessor * Formulation_{i,j} + \varepsilon_k \quad (3)$$

$$Acceptability\ score_{i,j,k} = \mu + Typology\ of\ assessor_i + Formulation_j + \varepsilon_k \quad (4)$$

where μ is the mean effect, *Assessor* is the assessor effect (29 assessors), *Formulation* is the formulation effect (7 levels), $Assessor * Formulation_{i,j}$ represents the interactions between formulations and assessors, $Typology\ of\ assessor_i$ is the class of assessor effect, and ε_k, the residual variance. Comparisons of means were made using the Newman-Keuls test ($p < 0.05$).

To complete the evaluation of product acceptability, the assessors were also asked to freely describe, in their own words, the organoleptic texture, flavour and aroma characteristics of the sausages.

4. Results and Discussion

4.1 Analysis of water and salt transfers

4.1.1 Effect on DFS chemical composition

DFS drying globally leads to a reduction in in-DFS water content due to water evaporation from the DFS surface and, in turn, to a fat and salt concentration that increases fat and salt content, respectively. We therefore ran chemical analysis of water content, salt content and fat content at four timepoints, i.e. Day 1, Day 7, Day 14 and Day 29, to track and trend the time-course of these parameters and check, at the end of drying, whether the objectives of reducing salt by 30% and fat by 60% are effectively achieved. To make results easier to discuss, the same chemical parameters were also measured on fresh lean pork meat (not dried) samples and are reported on the figures.

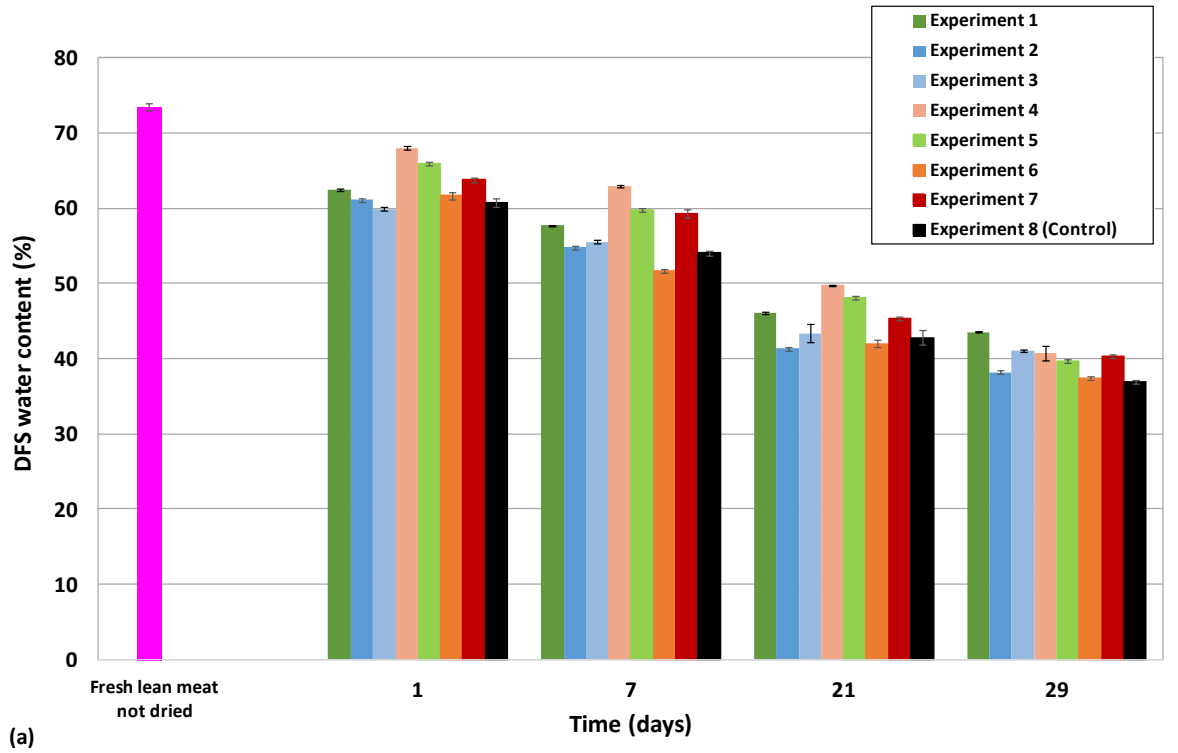
4.1.1.1 Time–course of in-DFS water content

Figure 7 shows the time-course evolution of in-DFS water content for the two fabrication series and confirms that in-DFS water content decreases globally with drying.

For the first fabrication series (Figure 7a), in-DFS water content decreases from initial values of 60%-68% at Day 1 to final values of 36%-43% at Day 29, with the lowest final water content values obtained for the two 21%-animal fat formulations (Experiments 2 and 8, the control) and for Experiment 6 corresponding to a high-fat but low-NaCl formulation (17.9% fat and 2.0% salt, respectively). Note that at Day 1, all in-DFS water content values are lower than the water content of undried fresh lean meat, probably as a result of adding pork backfat and NaCl to lean pork meat when preparing the meat batter. Indeed, at that time (Day 1), the higher in-DFS water contents were measured for the three formulations of the first fabrication series containing less than 12% animal fat. The highest value (68.1%) was even obtained for the lowest-fat formulation (Experiment 4 of Table 1).

For the second fabrication series (Figure 7b), whatever the drying time, the lowest in-DFS water losses clearly correspond to the three 21%-animal fat formulations (Experiments 9 to 11), with water content differentials higher than 7% at Day 1 and about 4% at Day 29 compared to the four other formulations that contain only 7% animal fat. Concerning the low-fat formulations, note too that slight differences emerged between the formulations containing 3% sunflower oil (SFO) (Experiments 13 and 15) for which the water content values are slightly higher than the low-animal-fat formulations that do not include SFO (Experiment 12 and 14). Moreover, we found no marked effect of KCl addition on in-DFS water content values can be found.

For the two fabrication series, it can be concluded that the respective proportions of lean meat and fat logically influence the in-DFS water content values. For an identical drying process, the highest water content values expressed in percentages are obtained for the low-fat products.



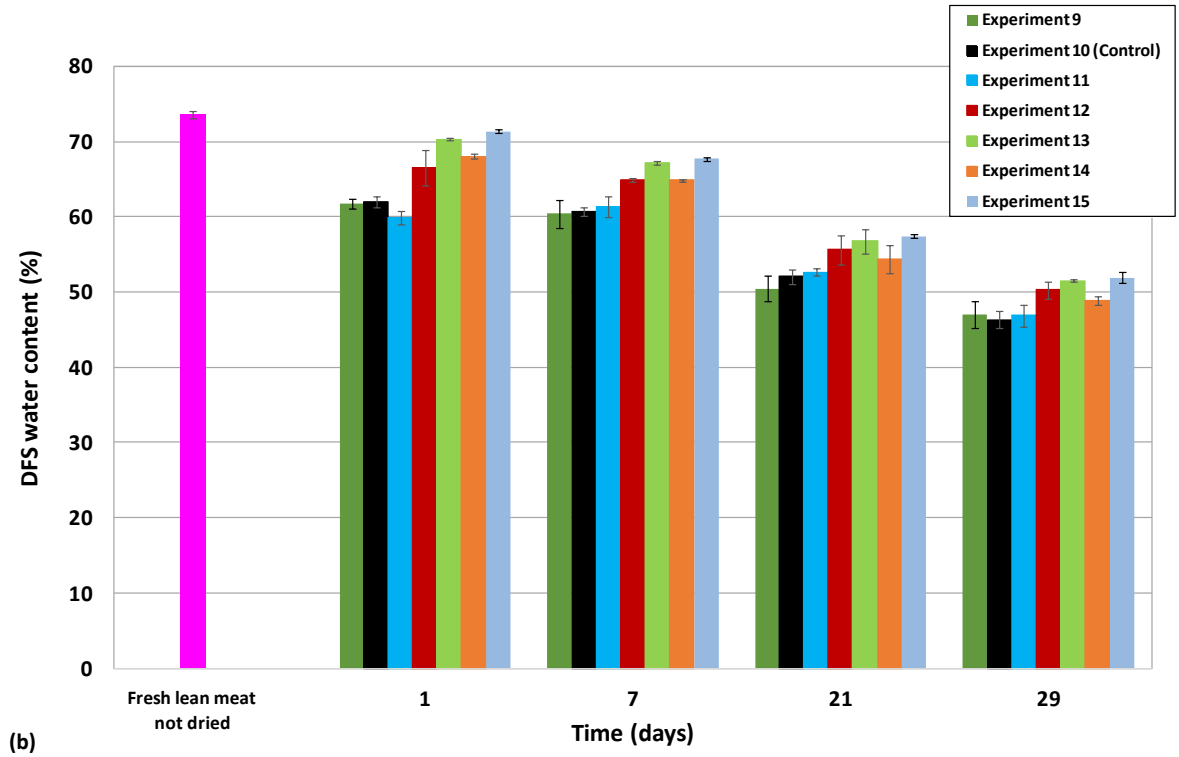


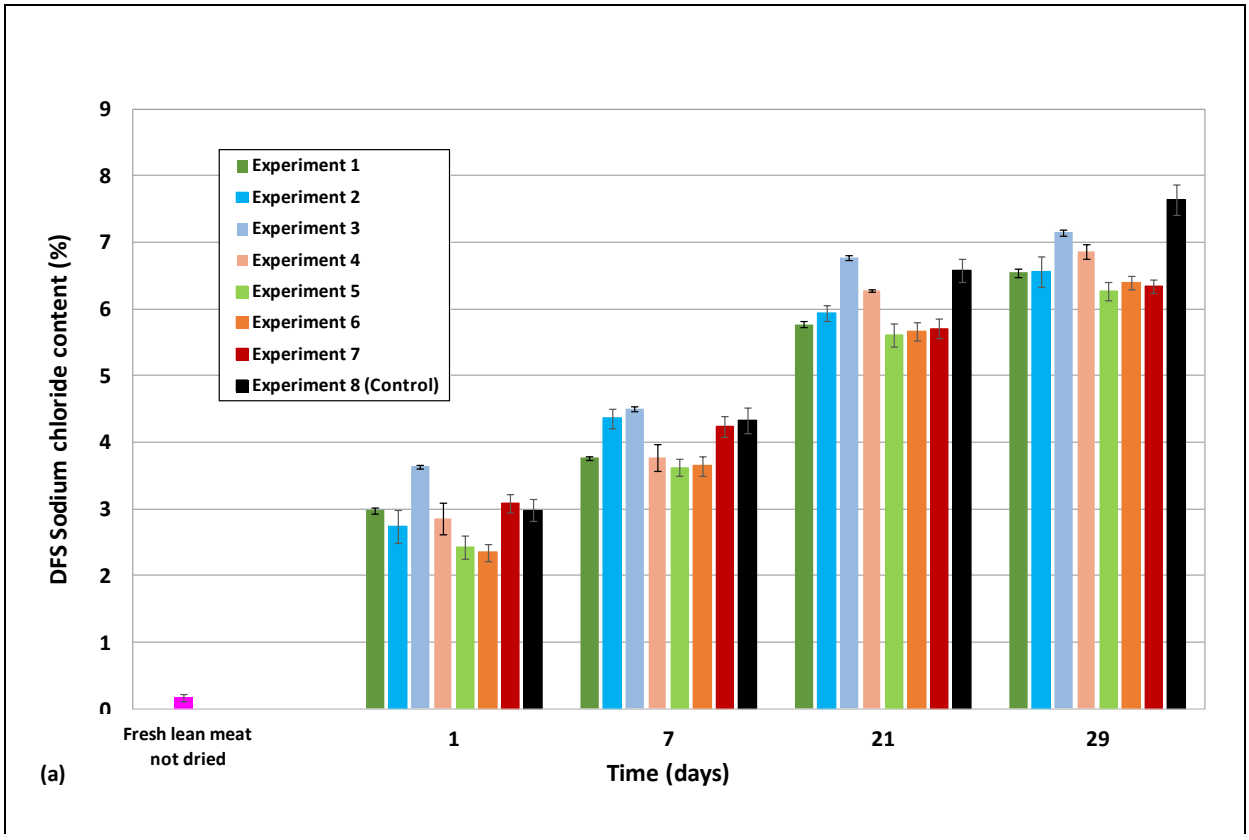
Figure 7. Time-course evolution of water content values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). Control fabrications are shown in black. Values are means ± SD of 6 independent determinations (n=6).

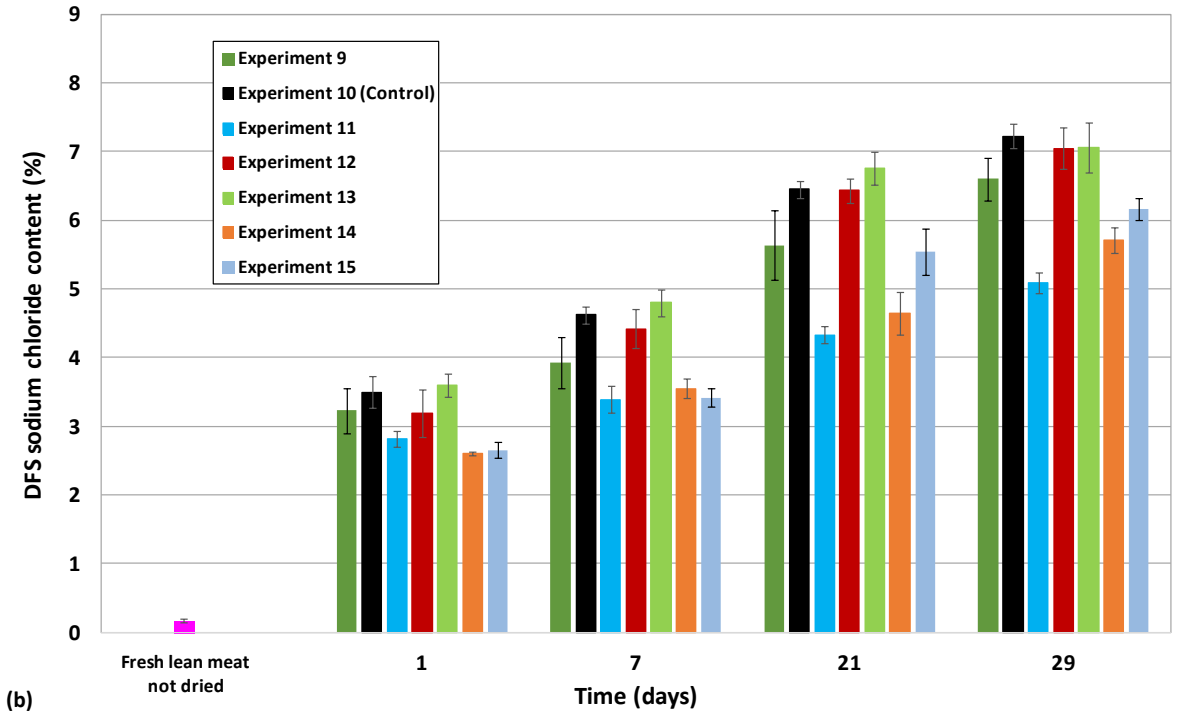
4.1.1.2 Time–course of in-DFS salt content

Figure 8 shows the time-course evolution of in-DFS salt (NaCl and KCl) content for the two fabrication series and confirms that in-DFS salt content increases globally with drying.

Analysis of Figure 8a charting the time-course evolution of NaCl content for the first fabrication series indicates that:

- Fresh lean pork meat naturally contains small amounts of sodium and chloride ions, leading to a 0.17% equivalent of NaCl content.
- At Day 1, the measured value of in-DFS salt content ranges from 2.3% to 3.6% depending on the experiment investigated, whereas the intended salt content corresponded to the levels of the Doehlert design: 2.0%, 2.4% and 2.8%. This implies that real salt content values are globally higher than intended values. Accurate analysis between real and intended salt content shows that discrepancies range from 0.18% (Experiment 8) to 0.83% (Experiment 3) depending on formulation, with a mean discrepancy of 0.42%. This underlines just how difficult it is to add exactly the intended amount of NaCl to meat batters with varying proportions of lean meat and fat, even at pilot scale level.
- At Day 29, the final values of NaCl content range from 6.2% to 7.6% depending on experiment and on the weight loss experienced by the DFS. The highest final NaCl content value was measured for the control (Experiment 8) which initially contained about 3.0% NaCl (Day 1) and whose final water content counted among the lowest (Figure 7a).





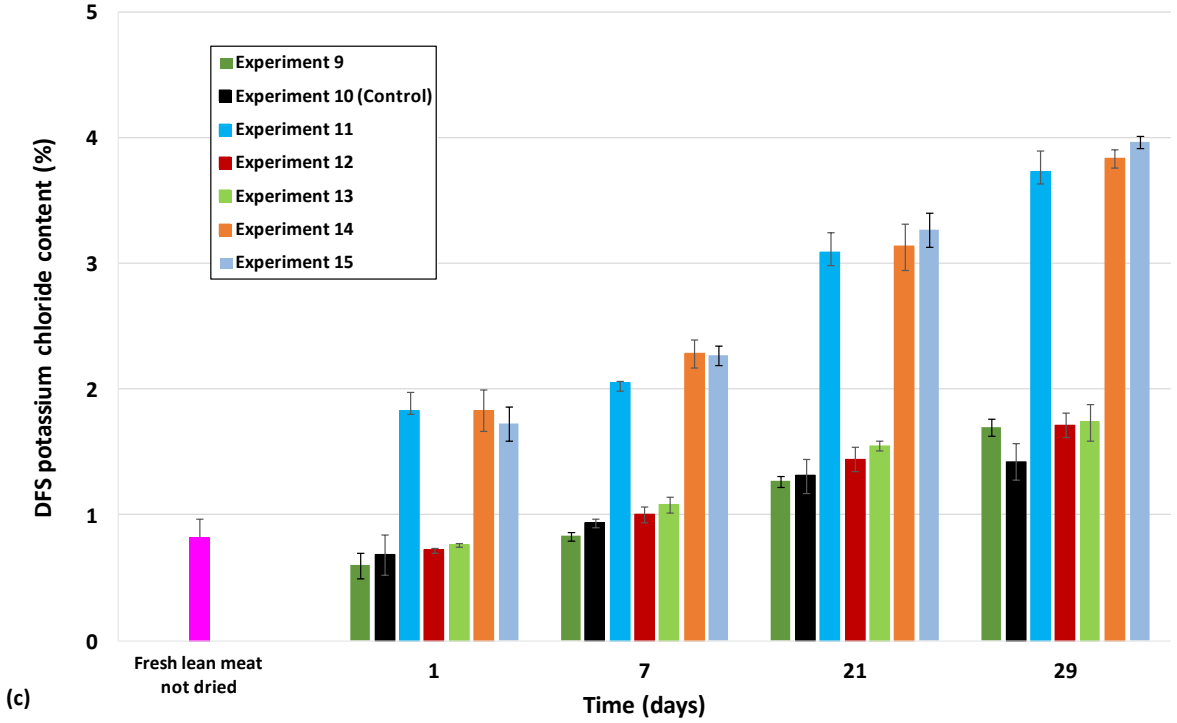


Figure 8. Time-course evolution of NaCl content measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15), and (c) KCl content measured in DFS corresponding to the second fabrication series. Control fabrications are shown in black. Values are means ± SD of 6 independent determinations (n=6).

Analysis of Figure 8b charting the time-course evolution of in-DFS NaCl content for the second fabrication series confirms the main findings of Figure 8a, namely:

- NaCl concentrates progressively with drying, whatever the formulation investigated.
- The real values of NaCl content measured at Day 1 are higher than the intended values, with measured values ranging from 2.6% to 3.6%. Here, the discrepancies between real and intended values of initial NaCl contents range from 0.39% to 0.81%, with a mean discrepancy of 0.62%. However, even if discrepancies exist, fortunately, the formulations with the higher intended salt content values (Experiments 9-10 and 12-13) were also the formulations that actually contained more NaCl at Day 1 than the others (Experiments 11 and 14-15).

At Day 29, there were strong variations in final NaCl content, with measured values ranging from 5.1% (Experiment 11) to 7.2% (Experiment 10, control). The highest final values of NaCl content were logically obtained for the four DFS formulations that initially contained 2.8% NaCl (from 3.2% to 3.6%, in reality). Figure 8b also shows that a 30% reduction in NaCl content can be roughly achieved with high-fat formulations (compare Experiment 11 with Experiment 10) but not reduced-fat formulations containing 7% animal fat, where the reduction in NaCl content reaches only 15% (compare Experiments 14 and 15 with control experiment 10).

In the specific case of the second fabrication series, we also experimentally tracked the time-course evolution of KCl content as 0.8% KCl had been added to the meat batter of some formulations in this series (Experiments 11, 14 and 15) as an NaCl substitute. These measurements are reported in Figure 8c, which clearly shows that:

- High values of equivalent KCl content were found in fresh lean meat and in the four formulations *a priori* not containing KCl at Day 1, with values of 0.6%-0.8% (Experiments 9-10 and 12-13).
- Consequently, the three formulations where 0.8% KCl wad added presented the highest values of KCl content at Day 1, with measured values ranging from 1.7% to 1.8% (Experiments 11, 14-15).
- At Day 29, the final concentration values peaked at 3.7%-3.9% for the three formulations initially added with KCl whereas for the four other formulations, final KCl content did not exceed 1.8%.

For the two series of DFS fabrication, it can be concluded that the measured salt content values (NaCl and KCl) at Day 1 were still higher than the intended values reported in Tables 1 and 2, probably as a result of the natural presence of sodium, chloride and potassium ions in lean pork meat and very probably as a result of real difficulties in perfectly adjusting the amount of salt added during the meat batter preparation as a function of the respective proportions of lean pork meat and fat. However, the formulations with the higher intended salt content values nevertheless contained more salt (NaCl and KCl) than the others, once the meat batters were prepared, thus *a priori* lowering the impact of this observed discrepancy on the results subsequently obtained.

4.1.1.3 Time–course of in-DFS fat content

Figure 9 charts the time–course of in-DFS fat content measured for the two series of DFS fabrication, and confirms that fat concentrates with drying.

What is remarkable in Figure 9a is that:

- Here, the fresh lean pork meat contains 7.3% fat.
- In a similar way to salt content, discrepancies emerge between measured in-DFS total lipid content and the intended fat content fixed by the Doehlert design, but the order is respected, meaning that the formulations with the highest intended fat contents have the highest measured total lipid contents, and vice-versa. For example, formulations corresponding to Experiments 2 and 8 (control) for which intended fat content was 21% really contained 19.0% and 20.6% total lipids, respectively, at Day 1. For the 17.9%-fat formulations (Experiments 3 and 6 of Table 1), the discrepancy in total lipid content was -0.8% and +0.2%, respectively. Moreover, 8.0%-total lipid content was measured in the specific case of Experiment 4 where 8.4% fat content was planned for the formulation. Finally, discrepancy in total lipid content ranged from -2.0% to +0.4% depending on experiment investigated.
- The formulations can be sorted as a function of their real total lipid content in the same order, from the beginning (Day 1) until the end (Day 29) of the drying process.

Figure 9b charts the time–course of measured total lipid content for the second fabrication series where three different types of lipid formulations were elaborated: 21% animal fat (Experiments 9 to 11), 7% animal fat without SFO (Experiments 12 and 14) and with 3% SFO (Experiments 13 and 15).

Figure 9b makes it possible to:

- clearly distinguish, from Day 1, the 21%-animal fat formulations (Experiments 9 to 11) from the 7%-animal fat formulations (Experiments 12 to 15). As previously, there were some discrepancies between measured total lipid content values and the intended fat contents reported in Table 2. Globally, fat content was underestimated in high-fat formulations from -1.9% to -1.1% and overestimated in low-fat formulations from 1.3% to 1.9%.
- unambiguously distinguish, from Day 21, the two 7%-animal fat formulations also containing 3% SFO from the other 7%-animal fat formulations not containing SFO. Measured total lipid content is 2%-3% higher at Day 21 for formulations containing SFO, and the difference is even greater at Day 29.
- see that final measured total lipid content values range from about 15% (Experiment 14) to 33% (Experiment 10), pointing to a potential 55% reduction in animal fat during DFS manufacture.

Concerning DFS fat content, in a similar way to salt content, there were discrepancies in total lipid content reaching 2% at most, again underlining the real difficulty in perfectly adjusting the amount of added fat during meat batter preparation. However fortunately, the formulations with the higher intended fat content values really contained more fat than the others, once the meat batters were prepared, thus *a priori* lowering the impact of this observed discrepancy on the results subsequently obtained.

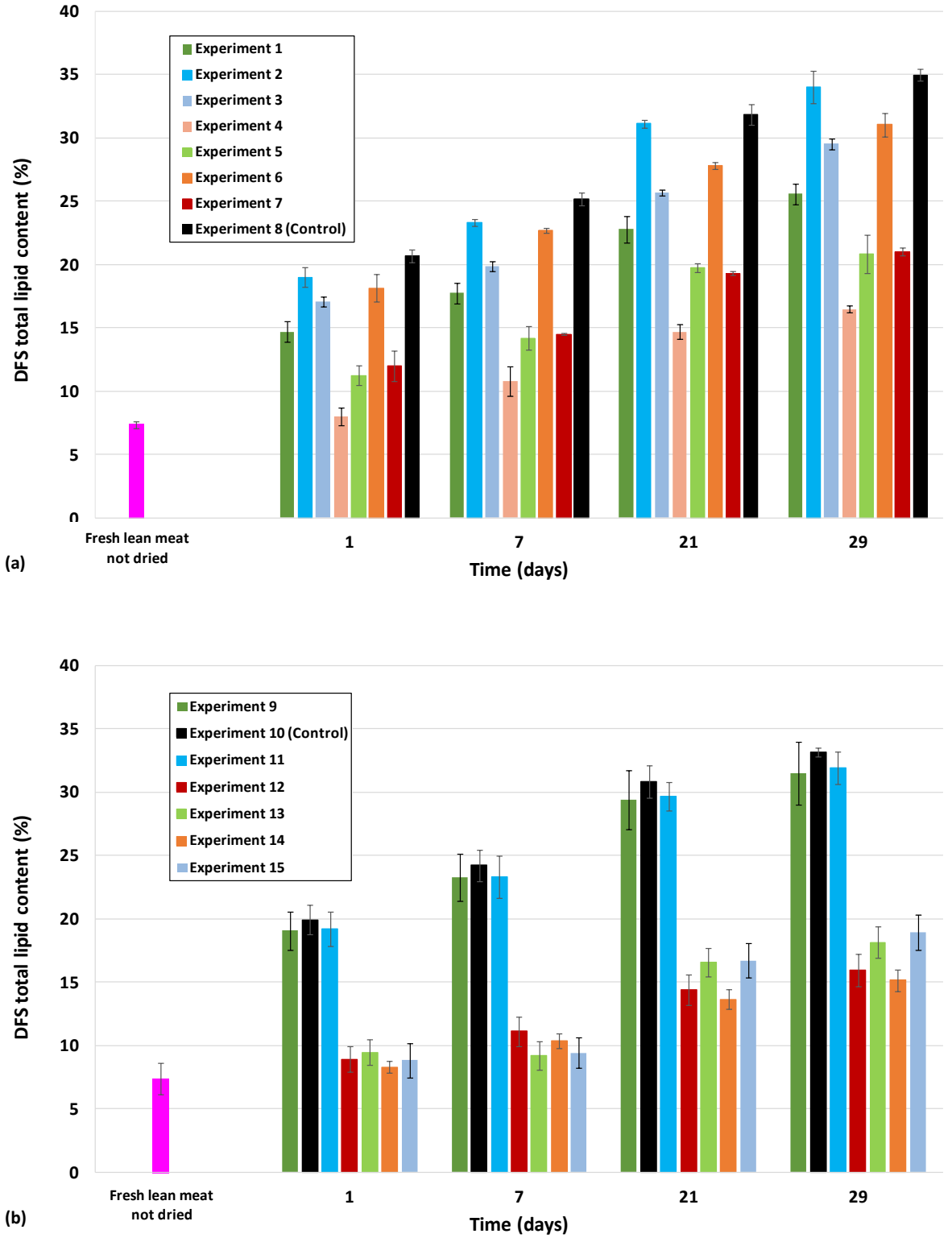


Figure 9. Time-course evolution of fat content values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). Control fabrications are shown in black. Values are means ± SD of 6 independent determinations (n=6).

4.1.2 Effect on in-DFS physicochemical parameters

This section presents the time-course pattern of physicochemical parameters measured for DFS of the two fabrication series, namely DFS weight loss, mean in-DFS a_w and mean in-DFS pH values. To make the results easier to interpret and the figures easier to read, a specific statistical treatment called hierarchical cluster analysis (HCA) was applied to all the measured raw values. HCA consists in clustering DFS formulations that lead to similar results on a given parameter, thereby creating classes of formulations. HCA was calculated based on the method of Ward, using STATISTICA 10-V2014 software. In addition, when a class of formulations is formed, the values of the parameter corresponding to the class are calculated, at each timepoint, by averaging the values of all the same-class formulations.

4.1.2.1 Time–course of DFS weight loss

Figure 10 shows the weight loss kinetics measured for the two fabrication series of Tables 1 and 2.

Applying HCA to the first fabrication series leads to the formation of three classes of formulations, as visible in Figure 10a. The first class, corresponding to the lowest weight loss (about 43% at Day 29), is composed of Experiments 1, 2, 3, 6 and 8, i.e. the formulations containing at least 14.7% animal fat. The two other classes of formulations that differ from the first class from Day 5 pool the three lowest-fat formulations that generate higher DFS water losses. These two classes are very close, differing only from Day 14, thus separating the three lowest-fat content formulations as a function of their relative NaCl content; the highest-salt formulation (Experiment 7) leads to lower weight loss (45.4% at Day 29) compared to the two other formulations (Experiments 4 and 5) that are less salty (2.4% and 2.0%, respectively) for which water loss reached 49% at the end of drying. Figure 10a shows that lean DFS globally loses more water than the fattiest DFS.

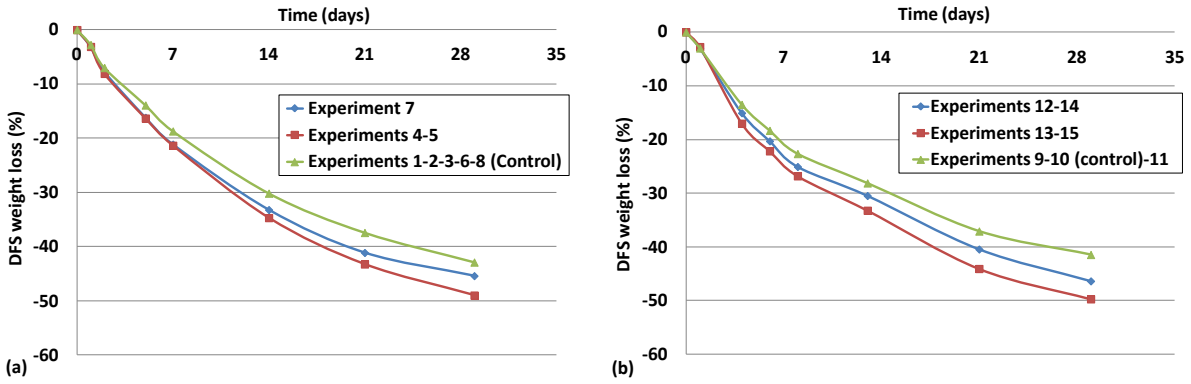


Figure 10. Time-course evolution of weight loss values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw weight-loss values in order to pool DFS formulations presenting similar patterns.

Applying HCA to the second fabrication series allows the 7 formulations of this series to be separated into three classes as a function of their respective fat content. The fattiest formulations (Experiments 9-11) form the class in which DFS water losses are the lowest (41.5% at Day 29). The other two classes are formed by the leanest formulations, i.e. those with 7% animal fat, but with higher water loss for the products also containing 3% SFO (49.7% *vs.* 46.4%, at Day 29). The behaviour difference in terms of DFS water loss between

the classes corresponding to 7%-animal fat formulations is visible from Day 4, i.e. during the fermentation stage, probably as a result of a weak oil loss in liquid form at that moment; indeed, it was noticed experimentally that the surface of DFS containing SFO remained oily throughout the first week of process (Experiments 13 and 15). Finally, Figure 10b shows no visible effect of salt content on HCA results.

Concerning DFS water loss, the present results based on HCA analysis showed a strong impact of animal fat content whatever the fabrication series, with about 8% variation in DFS water loss according to the formulations, and a moderate effect of salt content, exclusively for the first DFS fabrication series.

4.1.2.2 Time–course of mean in-DFS a_w values

Figure 11 charts the kinetics of mean in-DFS a_w values measured for the two fabrication series of Tables 1 and 2. Generally speaking, the drying process leads to a reduction in DFS water content and to a concentration of salt into the matrix, and thus to a decrease in a_w.

Applying HCA to the first fabrication series leads to the formation of three classes of formulations, visible in Figure 11a. The class corresponding to the lowest mean values of in-DFS a_w (with variations from 0.958 at Day 1 to 0.89 at Day 29) is formed by Experiments 3, 7 and 8, i.e. the three highest-NaCl formulations containing at least 11.6% animal fat. The class corresponding to the highest mean in-DFS a_w values, with variations from 0.963 at Day 1 to 0.90 at Day 29, is formed by Experiments 4, 5 and 6, i.e. the two lowest-NaCl formulations of Table 1 (Experiments 5 and 6) and the lowest-fat formulation of Table 1 containing only 8.4% animal fat (Experiment 4). The third class presents an intermediate pattern in terms of time–course of mean in-DFS a_w, with values ranging from 0.960 at Day 1 to 0.90 at Day 29. Experiments 1 and 2 which contain moderate NaCl content (2.4%) and a fairly high (>14.7%) animal fat content form this third intermediate class. Note that all the final mean in-DFS a_w values are below 0.92 and that a_w values below 0.92 are considered safe in terms of *Listeria monocytogenes* growth capacity (European Commission regulation CE 2073/2005). At Day 1, the differences in mean initial values of DFS a_w can be directly explained by the amount of salt added in the meat batters. Indeed, the formulations containing 2.8% NaCl and corresponding to Experiments 3, 7 and 8 presented the lowest initial mean a_w value (0.958). The 2%-NaCl formulations (Experiments 5 and 6) presented the highest initial mean a_w value (0.963), and the 2.4%-NaCl formulations (Experiments 1 and 2) presented intermediate initial mean a_w values (0.960). The fact that Experiment 4, which contains 2.4% NaCl, belongs to the class presenting the highest mean of DFS a_w values and not to the intermediate class is almost certainly explained by a salt dilution effect on the lean part of the meat batter since only a relatively low amount of animal fat (8.4%) was added.

Applying HCA to the second fabrication series allows the 7 formulations of this series to be separated also into three classes as a function of their respective salt and fat content and the type of salt (NaCl or KCl) used. The 2.8%-NaCl formulations also containing 21% animal fat (Experiments 9-10) form the class in which mean in-DFS a_w values decrease the fastest, with variations from 0.957 at Day 1 to 0.885 at Day 29. The class presenting the highest mean in-DFS a_w values pools the three formulations in which partial substitution of NaCl by KCl was applied (Experiments 11, 14 and 15 of Table 2), thus confirming that KCl has a lower a_w depressor effect than NaCl. Between these two, the intermediate class in which mean in-DFS a_w values range from 0.959 at Day 1 to 0.888 at Day 29, is formed by Experiments 12 and 13 that contained 2.8% NaCl like class I but only 7% animal fat plus 3% SFO for experiment 13 (salt dilution effect on the lean part of the meat batters). Like for the first fabrication series, all final mean in-DFS a_w values were comfortably below the 'safe' threshold value of 0.92. Note that, in Figure 11b, the mean initial values of DFS a_w

are very close for the three classes because 2.8% salt was used in all of the formulations, i.e. either 2.8% NaCl or 2.0% NaCl plus 0.8% KCl. However, a very slight difference can be seen for the two high-fat formulations that contain 2.8% NaCl (Experiments 9 and 10), where the mean initial a_w value is logically slightly lower than for the other formulations (0.957 *vs*. 0.959).

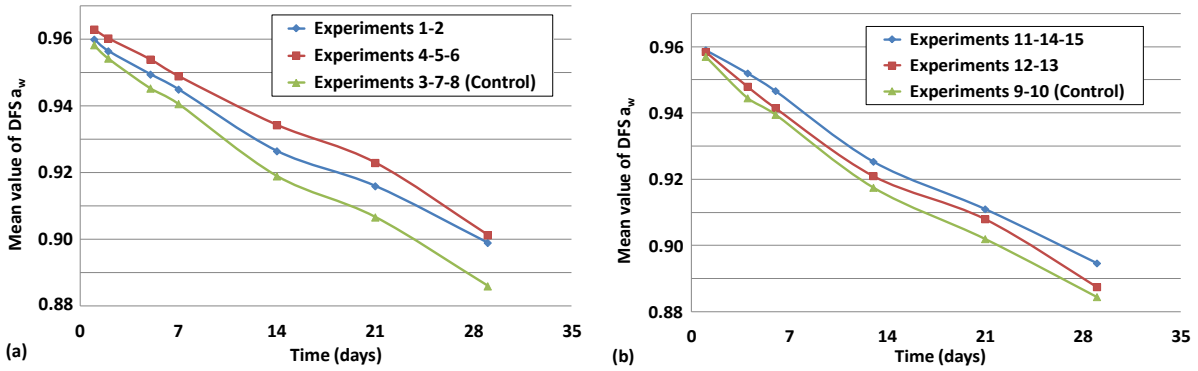


Figure 11. Time-course evolution of mean a_w values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw a_w values in order to pool DFS formulations presenting similar patterns.

Concerning DFS water loss, whatever the fabrication series, the HCA analysis-based results presented here logically showed a strong impact of salt content, an impact of type of salt (NaCl or KCl), and also an effect of fat content. Indeed, modifying the fat content of the meat batter modifies the salt concentration in the lean part of the batter, and thus the a_w value. From an a_w perspective, reducing fat content in DFS provokes the same drop on a_w as reducing salt content. So, binary reductions in DFS fat and salt content may prove detrimental from a safety standpoint if the products are not sufficiently dried.

4.1.2.3 Time–course of in-DFS pH values

Figure 12 charts the kinetic of in-DFS pH values measured for the two fabrication series of Tables 1 and 2. Both cases produced a normal time-course of pH values—except experiment 9 of Table 2—with a strong decrease in pH values during the first week of process, from 5.9 at Day 1 to a minimum of about 5.0 at Day 7, corresponding to intense acidification due to the action of the lactic acid bacteria (LAB) added during DFS manufacture. Beyond Day 7, pH values increase progressively until the end of the drying process as a result of an array of phenomena including a strong decrease in LAB acidifying action due to the depletion of sugar substrate, the transformation of lactic acid into other chemical substances and/or the production of alkaline molecules due to proteolytic mechanisms. Except for Experiment 9 where no flavouring (neither garlic or pepper) was added during DFS manufacture, all the other pH values obtained can be considered as acceptable as they mirror the pH values classically found in DFS.

HCA applied to the in-DFS pH values of the first fabrication series led to the formation of two distinct classes differentiated according to NaCl content of the formulations (Figure 12a). Lower pH values were obtained for the two lowest-salt formulations, i.e. Experiments 5 and 6 that contain only 2.0% NaCl, probably due to more intense LAB activity. The second class pooled all the other formulations that contain at least 2.4% NaCl. The difference between the two classes resides in the fact that a more intense

acidification is observed for the two lowest-salt formulations during the first week of process (with 0.10 pH unit less at Day 7); this discrepancy persists when the pH values increase over the next two weeks of process, before growing slightly stronger during the last week of process to peak at 0.15 pH units at Day 29 (Figure 12a). However, all these pH values, especially the lowest and final values, are fully representative of what happens classically when French DFS are manufactured.

Figure 12b shows the three distinct classes of formulations formed when HCA was applied on the second fabrication series. As stated earlier, the lack of flavouring in Experiment 9 led to an abnormal time-course of pH due to an insufficient acidification phase during the first week and a minimum pH of 5.5. This over-high pH value at the end of the acidification phase may prove detrimental for DFS shelf-life and even food safety, as low pH values are one of the barriers against spoilage by microbial growth or pathogen growth along with a_w, temperature and added salt. It thus appears that the LAB action was seriously disrupted by not adding flavouring, even if no difference appeared when measuring mean a_w value for the DFS of Experiment 9 (Figure 11b). The two other classes show similar patterns of pH evolution and only differ after the acidification phase from the moment that pH values begin to increase, with a stronger increase in pH for the lowest-fat formulations (Experiments 12-15) than the high-fat and flavoured formulations (Experiments 10-11), reaching a difference of 0.14 pH units at Day 29. Nevertheless, all the pH values of these last two classes of formulations are fully acceptable.

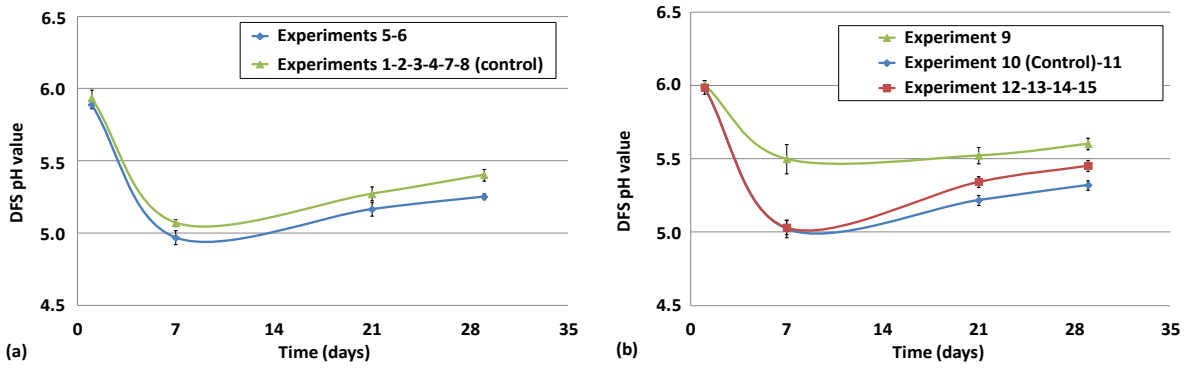


Figure 12. Time-course evolution of pH values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series of (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw pH values in order to pool DFS formulations presenting similar patterns.

Concerning in-DFS pH values, whatever the fabrication series, the HCA analysis-based results presented here showed normal time-course of pH value, with two distinct phases: strong acidification during the first week of process followed by a progressive increase in pH value, except in the non-flavoured formulation (Experiment 9). This therefore highlights a strong impact of flavouring on time-course of pH values, an impact of salt content, and a moderate effect of fat content. HCA analysis found no discernible effect of type of salt (NaCl or KCl).

4.1.3 MBRA results

Purpose-built lab-scale micro-bioreactors (MBRA) were used to dry one DFS per drying cell in each MBRA. As we had 5 MBRA systems, we chose five raw sausages corresponding to five different formulations of the first fabrication series, i.e. Experiments 2, 4, 5, 7 and 8

(control). Moreover, MBRA drying trials were performed at the same time as drying first series DFS in the ADIV pilot drying room.

Figure 13 shows the kinetics of DFS water loss estimated from measurements made in MBRA systems over 35 days. In-depth analysis of Figure 13 shows that the lowest DFS weight loss was obtained for the control (Experiment 8), then for Experiments 2, 7, 5 and finally 4. Globally, the lowest weight losses were measured for the 21%-animal fat formulations, then for the 11.6%-animal fat formulations (Experiments 7 and 5), and finally for the 8.4%-animal fat formulation. For the same animal fat content, DFS weight losses were higher for the formulations containing less NaCl (Experiment 8 *vs*. Experiment 2 and Experiment 7 *vs*. Experiment 5). At constant salt content, DFS weight losses were higher for the formulations containing less animal fat (Experiment 8 *vs*. Experiment 7 and Experiment 2 *vs*. Experiment 4). Reducing fat content and salt content thus leads to increased DFS water losses when the same drying conditions are applied.

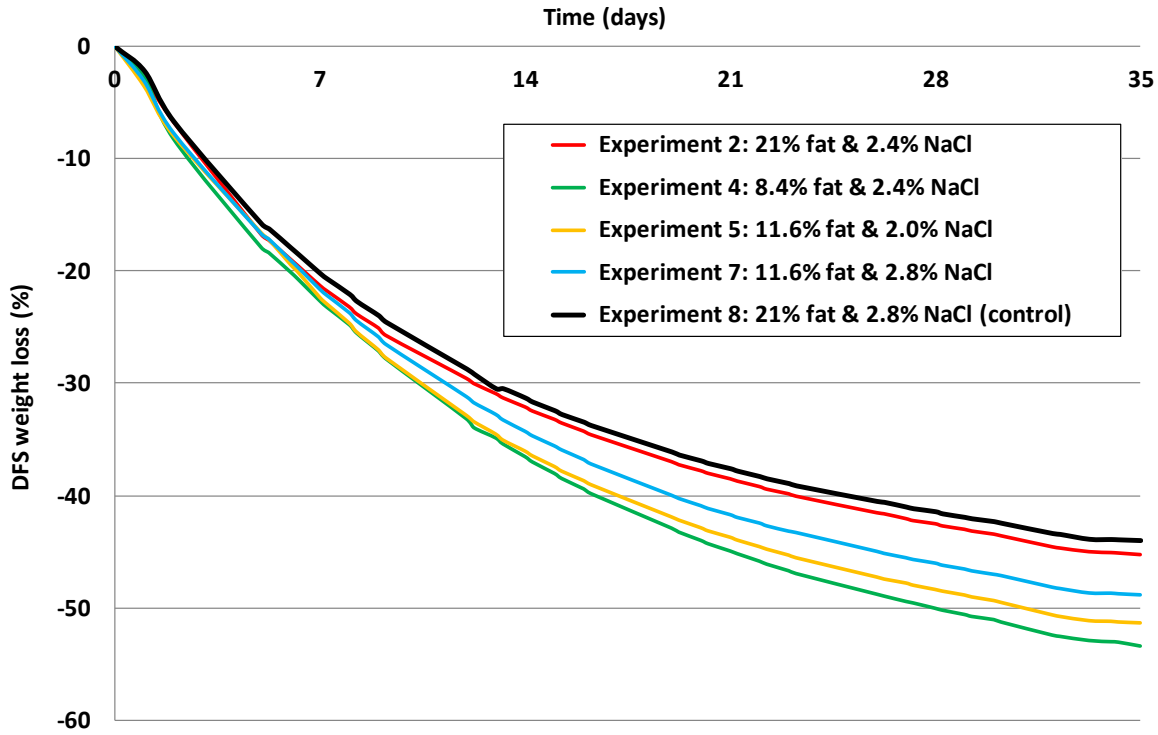


Figure 13. Time-course evolution of weight loss values measured in dry-fermented sausages (DFS) using the micro-bioreactors (MBRA) for 5 formulations representative of the first fabrication series (Experiments 2, 4, 5, 7 and 8).

The results presented in Figure 13 perfectly agree with the results of Figure 10a which corresponds to the weight losses measured in the ADIV pilot drying room for the same formulations, thus proving that the drying conditions in the MBRA systems were perfectly adjusted. Figure 13 shows little difference in terms of DFS weight loss between the two 21% animal-fat formulations (Experiments 8 and 2) and between Experiments 5 and 4. In Figure 10a, Experiments 8 and 2 belong to the same 'lowest weight loss' class, and Experiments 5 and 4 belong to the same 'highest weight loss' class. In both cases, Experiment 7 presented intermediate weight loss compared to the other formulations.

The MBRA drying trials were completed by measuring microbial flora activity through the quantification of CO_2 production by regularly placing each DFS in a hermetically-sealed

plastic box for 120 min. To our great surprise, no gas production was ever recorded, whatever the DFS formulation or drying time. This means that microbial flora has very low activity in DFS and no effect on the water transfers (evaporated water flux and surface a_w) occurring inside and thus from core to surface of the sausage. Indeed, in cheese, we recently highlighted a close interaction between surface microbial flora development and the water transfers occurring both from cheese core to surface and at the surface itself (Le Page et al., 2012). The growth of microbial flora gave rise to a mechanism that extracted water from the core to the cheese surface; this diffusion-based internal water flux would have been higher than the water flux evaporating from the cheese surface, thus ultimately re-humidifying the cheese surface. Apparently, this kind of mechanism was not reproduced during DFS drying, thus probably explaining why no marked increase in evaporated water flux at the DFS surface or global water losses were visible in Figure 13. Further experiments should be performed with raw sausages made with natural casings rather than artificial casings as here in order to confirm or disconfirm this finding.

The main conclusion from the MBRA drying trials is that surface microbial flora growth did not modify water transfers inside and at the surface of the product, in contrast to what had recently been observed on two types of cheese. This means that the water transfers (evaporated water flux, a_w and weight loss) in the dried products investigated here were directly induced by the drying conditions applied. That underlines the value of building a numerical function making it possible to calculate local a_w value as a function of local water content, local NaCl content and local fat content anywhere in a DFS.

4.1.4 DFS isotherm sorption

Given the MBRA results, and based on the chemical composition and a_w measurements made on the DFS samples, we decided to build a numerical function linking a_w to water content, NaCl content and fat content. This numerical function is the sorption isotherm presented in Figure 14 and given by equation (5):

$$a_{w,DFS} = [-0.4553 \,.\, (X_{NaCl}^{Water})^2 - 0.5242 \,.\, (X_{NaCl}^{Water}) + 0.999] * \left[0.991 \,.\, e^{(-0.0204 \,.\, (X_{Water}^{Protein})^{-1.96})}\right] \quad (5)$$

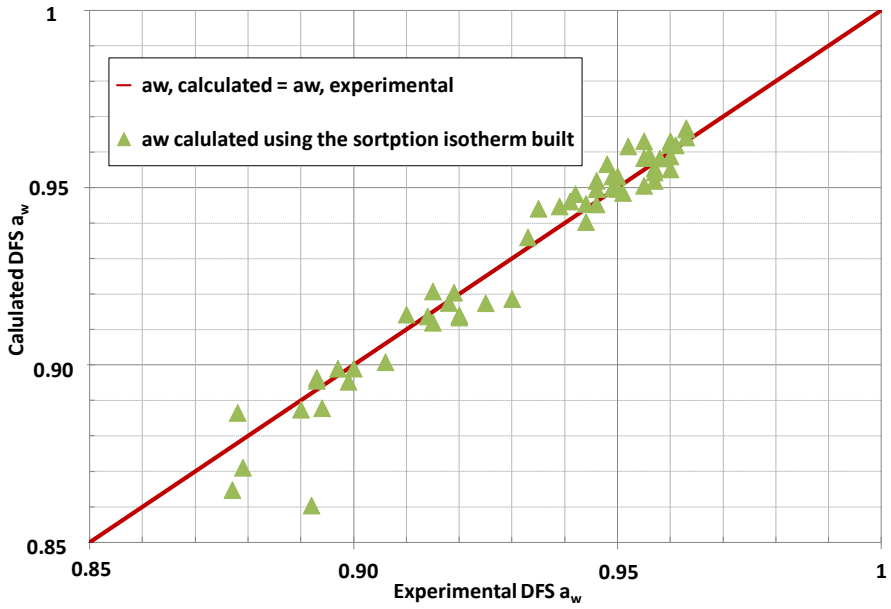


Figure 14. Dry-fermented sausage (DFS) sorption isotherm built from analysis of DFS chemical composition and a_w measurement and from the model of Rougier et al. (2007).

The equation was fitted on 56 measurement points from the relation proposed by Rougier et al. (2007) in the case of fatty and salty gelatine gels. Using this equation implies determining NaCl content as a function of water content (X_{NaCl}^{Water}) and water content as a function of protein content ($X_{Water}^{Protein}$). This sorption isotherm will be then implemented in a numerical finite-element-based model of water and salt transfers in order to simulate and visualize the distribution of salt content, water content and thus $a_{w,DFS}$ in DFS geometries.

4.2 Time-course of in-DFS biochemical parameters

This section presents the time-course of the biochemical parameters measured for DFS in the two fabrication series, namely DFS proteolysis, lipolysis, lactic acid content, lipid oxidation and protein oxidation. Like for the physical-chemical parameters, to make the results easier to interpret and the figures easier to read, we ran a HCA on all the measured raw values by grouping all DFS formulations leading to similar results on a given parameter into the same class.

4.2.1 Proteolysis

Figure 15 charts the time-course of DFS proteolysis index (PI) measured for the two fabrication series of Tables 1 and 2, charted as HCA-grouped classes of formulations. All 15 formulations produced the same pattern of behaviour in terms of PI time, namely:

- A 2.9%-3% PI value at Day 1 resulting from the action of the proteolytic enzymes present in the products that are fully active at a temperature of 24°C, which is the air temperature imposed during the fermentation stage.
- An increase in PI values until Day 21, with values peaking at 6.5% for the first fabrication series and 5.5% for the second fabrication series but with a noticeable reduction in PI velocity from Day 7, probably due to a non-optimal pH value for proteolytic enzyme activity due to the intense lactic acidification occurring at that moment in time, as shown in Figure 12.
- Beyond Day 21, very surprisingly, PI values decreased for all classes of formulations. This is difficult to explain. It could be due to a problem with the technique used to measure PI that underestimated amino acid and peptide contents in DFS samples further to the oxidation of some amino acids, or else to the formation of biogenic amines. However, using the classical PI measurement method consisting in calculating the percentage ratio of non-protein nitrogen content to total nitrogen content, very recent measurements in some DFS samples confirmed this decrease in PI values over the last week of the drying process. This ultimately tends to prove that some end-products of proteolysis had disappeared and so could not be detected by the measurement techniques used, these end-products being probably consumed by the microorganisms in the DFS at that moment.

For the first fabrication series, Figure 15a shows that HCA formed three classes of formulations. In-depth analysis of results highlighted that the formulations are perfectly classified as a function of their respective NaCl content, thus further confirming the inhibitory effect of salt content on proteolytic enzyme activity. Indeed, the first class that leads to the lowest PI values is formed by the three 2.8%-NaCl formulations (Experiments 3, 7 and 8); the second class by the 2.4%-NaCl formulations (Experiments 1, 2 and 4) and the third class by the 2.0%-NaCl formulations (Experiments 5 and 6). At Day 21, PI values were 5.2%, 6.3% and 6.7%, respectively, according to class or salt content considered. Moreover, no marked effect of fat content is visible in Figure 15a. Nevertheless, when comparing Experiments 5 and 6 that belong to the same class, fine-grained analysis of the raw results reveals that proteolysis was slightly more intense in Experiment 5 than Experiment 6, with Day-21 PI values of 6.8% and 6.6%, respectively,

probably as a result of a lower fat content (11.6% *vs.* 17.9%) that provokes a more marked salt dilution and thus a lower salt concentration in the lean part of the meat batter that contains the greater quantity of lean pork meat (i.e. Experiment 5).

For the second fabrication series, Figure 15b shows that HCA formed four classes of formulations, but with lower between-class differences in PI evolution patterns compared to Figure 15a, possibly because salt content differed little between formulations ('2.8% NaCl' or '2.0% NaCl, plus 0.8% KCl') in this second series. For Experiments 9 to 15, PI values did not exceed 5.5%.

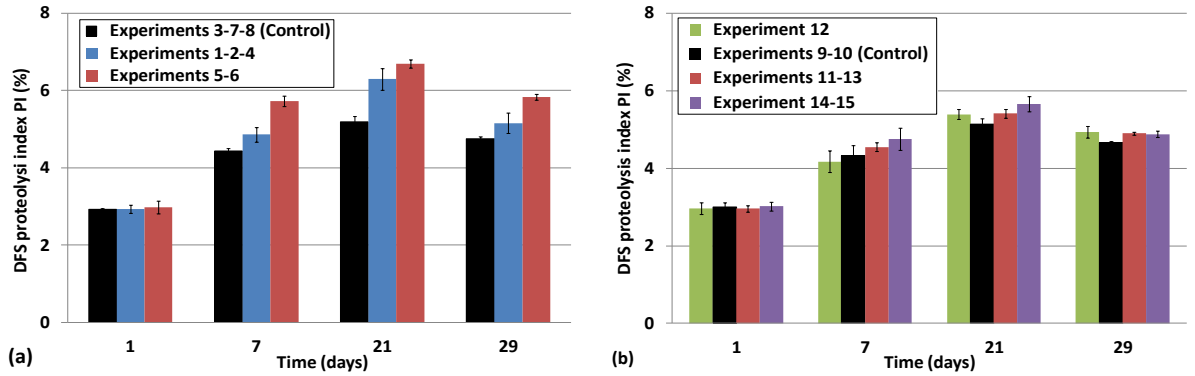


Figure 15. Time-course evolution of proteolysis index (PI) values measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw PI values in order to pool DFS formulations presenting similar patterns.

These results confirmed that proteolysis in DFS was mainly governed by their salt content. Reducing the salt content increases proteolysis, which can be detrimental for the final texture of the end-products. This critical point warrants checking via the texture profile analysis on the DFS samples.

4.2.2 Lipolysis

Figure 16 shows the lipolysis values determined on DFS samples from the two fabrication series at Day 29 via the determination of an 'acidity' value. For the two series, we found differences in acidity values between the various formulations.

For the first fabrication series, two distinct classes were formed as a function of animal fat content, regardless of NaCl content. One class pools the four formulations for which fat content is at most equal to 14.7%, and the other class pools the 17.9% animal fat and 21% animal fat formulations. Mean acidity value was significantly higher for the formulations containing more than 17% animal fat, at 9.8 *vs.* 7.6 mg KOH per g of DFS fat (Figure 16a).

For the second fabrication series, Figure 16b shows three distinct classes with two classes counting only one formulation. Indeed, measured acidity was different in Experiment 12 and Experiment 11, whereas all the other formulations belonged to the same class with a mean acidity value of 10.1 mg KOH per g of DFS fat. Note that the acid value of the two controls (Experiment 8 for series I and Experiment 10 for series II) were exactly the same (at 9.7 mg KOH per g of DFS fat). Moreover, the fact that a lower acid value (7.4 mg KOH per g of DFS fat) was found for Experiment 12 may be explained by its combination of high NaCl content (2.8%) and low animal fat content (7.0%). Regarding Experiment 11, the highest acidity value measured (12.3 mg KOH per g of DFS fat) may be attributable to its

high animal fat content (21%) associated to the presence of 0.8% KCl, unlike Experiments 9 and 10 that contain only 2.8% NaCl. This difference in terms of formulation leads to a difference in mean in-DFS a_w value, as shown in Figure 11b, with slightly higher a_w values for Experiment 11 compared to Experiments 9 and 10.

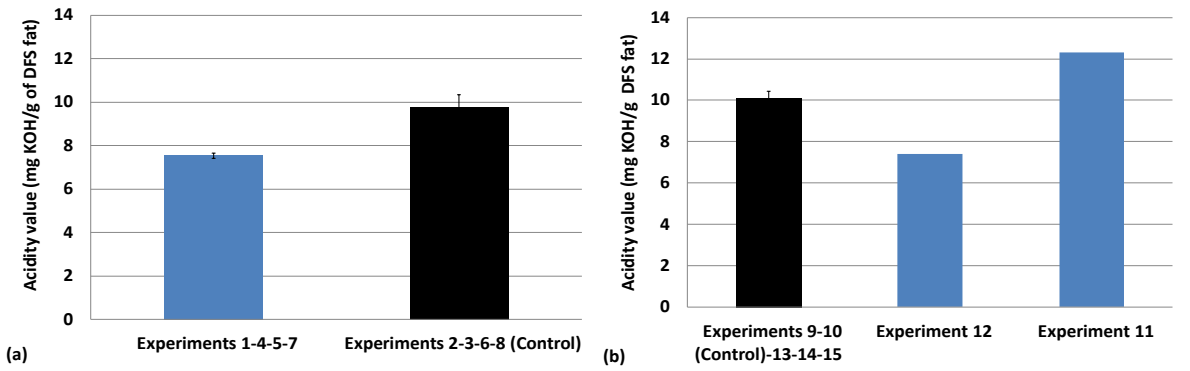


Figure 16. Lipolysis quantification in dry-fermented sausages (DFS) at Day 29 for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw values in order to pool DFS formulations presenting similar patterns.

To conclude on lipolysis, the data seems to suggest that the intensity of this biochemical phenomenon is mainly dependent on fat content, but further quantification of lipolysis is needed, maybe using another experimental method than determination of acid value, to definitively conclude on the effect of reducing salt and fat content on DFS lipolysis.

4.2.3 Lactic acid content

Figure 17 charts the time course of lactic acid content for the two fabrication series. Differences in lactic acid content were found between all formulations, but lactic acid content globally increases with drying, from about 1 g per kg of meat to at most 4.8 g per kg of meat for Experiments 5 and 6, i.e. the two lowest-salt formulations. The fact that lactic acid content increases continuously whereas pH time-course presented two distinct phases with a pH rise after Day 7 means that, from that moment on, the acidifying effect due to lactic acid production does not counterbalance the effect resulting from the production of alkaline molecules.

For the first fabrication series, HCA led to three distinct classes of formulations and globally classified formulations as a function of NaCl content. As previously indicated, from Day 7 of process, the highest lactic acid contents were measured for the 2.0%-NaCl formulations, thus explaining why a more intense acidification resulting in lower pH values was observed in Figure 12a. Moreover, a slight difference in lactic acid production is visible in Figure 17a between Experiment 8 and Experiments 1, 2, 3, 4 and 7, but with no marked impact on pH time-course; Figure 12a groups these 6 formulations into the same class.

For the second fabrication series, the main messages of the results charted in Figure 17b are as follows:

- In terms of lactic acid production, Experiment 9 is atypical with a non-progressive increase and very weak production during the fermentation stage, thus explaining the atypical time-course of pH depicted in Figure 12b for this formulation.

- The highest lactic acid production, at 4.5 g per kg of meat, is found in Experiments 10 and 11, i.e. the two formulations containing 21% animal fat and 2.8% salt. Note that the quantity of lactic acid produced in Experiment 10 (control) is the same as that produced in Experiment 8, i.e. the control of the first fabrication series.
- For Experiments 12 to 15, lactic acid production is also progressive but lower than in Experiments 10 and 11, especially after the fermentation stage. In response to this low lactic acid production after Day 7, pH values logically increased faster for these four formulations, as shown in Figure 12b.

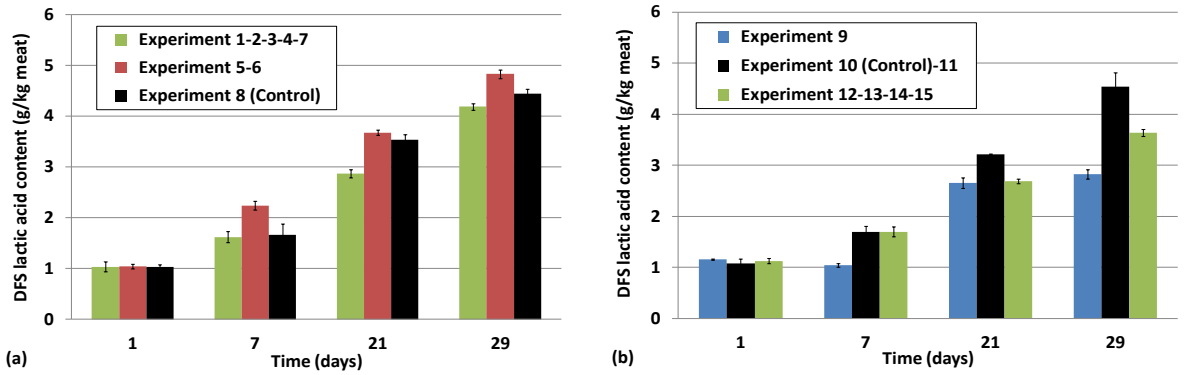


Figure 17. Time course evolution of lactic acid content measured in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw values in order to pool DFS formulations presenting similar patterns.

To conclude on lactic acid content, acid production rates fit perfectly with pH time-course values.

4.2.4 Lipid oxidation

Lipid oxidation in DFS samples was quantified by determination of (1) TBARS values and (2) HSB values. These two methods for assessing lipid oxidation in meat are complementary as different lipid oxidation dynamics lead to various end-products being produced.

4.2.4.1 TBARS values

Figure 18 charts the time-course of lipid oxidation quantified through the determination of TBARS values. For the two fabrication series, TBARS values decreased as a function of time, which is not a logical pattern, indicating that the TBARS quantification method is ill-suited to accurate assessment of lipid oxidation in DFS.

For the first fabrication series, the 8 formulations were classified into two groups as a function of fat content, with the highest TBARS values corresponding to formulations containing at least 17.9% animal fat (Experiments 2, 3, 6 and 8) and the lowest TBARS values corresponding to formulations containing no more than 14.7% animal fat. Higher fat content means higher lipid oxidation (Figure 18a).

For the second fabrication series, results were similar to the first series, with a lower lipid oxidation for the two lowest-fat formulations containing only 7.0%-animal fat and higher TBARS values for the five other formulations containing either 21%-animal fat or 7%-animal fat plus 3% SFO. Note that adding 3% vegetable oil to formulations containing 7% animal fat provoked an increase in lipid oxidation equivalent to what happened for the experiments

led with 21% animal fat (Figure 18b). This may be because oleic sunflower oil is rich in polyunsaturated fatty acids (PUFAs), which are known to be very sensitive to oxidation.

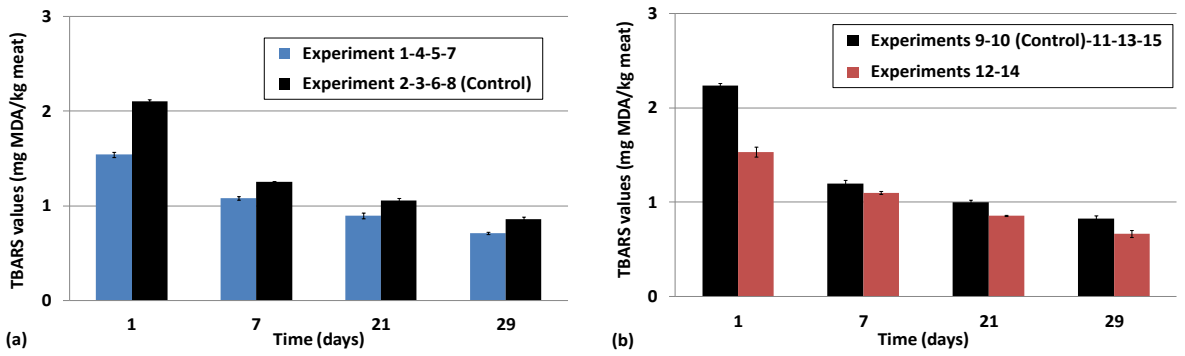


Figure 18. Time-course evolution of lipid oxidation quantified by determining TBARS values in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw TBARS values in order to pool DFS formulations presenting similar patterns.

4.2.4.2 Hydrosoluble Schiff Base values

Figure 19 charts the time-course of lipid oxidation quantified by determining hydrosoluble Schiff base (HSB) values. For the two fabrication series, contrary to the previous TBARS method, HSB values increased observably as a function of time, which is logical, meaning that lipid oxidation increases with time. The HSB quantification method therefore appears more appropriate than the TBARS method for accurate assessment of lipid oxidation in DFS. In addition, for the two fabrication series, the various formulations are globally classified as a function of their fat content; a higher fat content means higher HSB values and thus higher lipid oxidation.

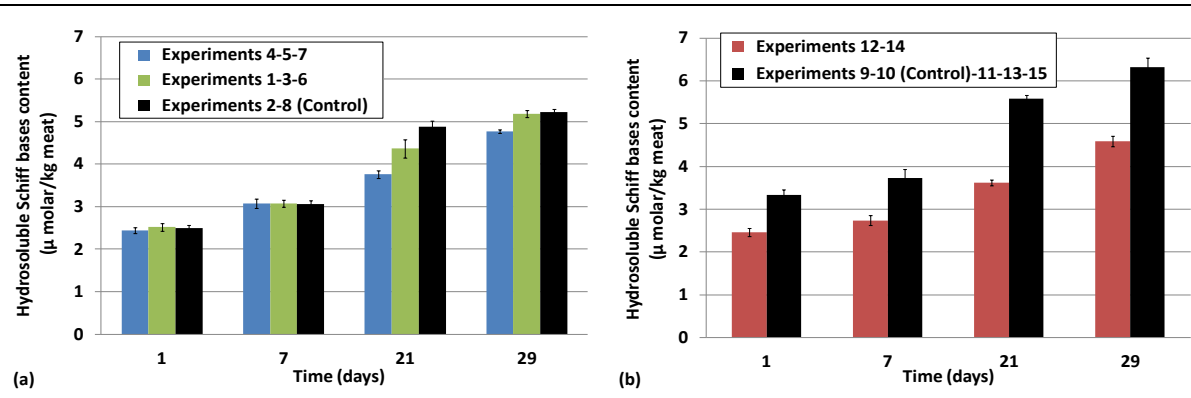


Figure 19. Time-course evolution of lipid oxidation quantified by determining hydrosoluble Schiff base (HSB) values in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw HSB values in order to pool DFS formulations presenting similar patterns.

For the first fabrication series, the 8 formulations were classified into three distinct classes: the first class formed of the three formulations containing at most 11.6% animal fat (Experiments 4, 5 and 7), the second class formed of the three formulations for which

animal fat content ranged from 14.7% to 17.9% (Experiments 1, 3 and 6), and the third class gathered the two 21%-animal fat formulations (Experiments 2 and 8). Figure 19a shows that these three classes differ in terms of lipid oxidation, especially at Day 21, less so at the end of DFS drying (Day 29), and not really early on in the process (Days 1 and 7).

For the second fabrication series, the seven formulations were classified into just two classes: a first class that leads to the lowest HSB values that was formed by the two leanest formulations containing only 7% animal fat (Experiments 4 and 6), and a second class covering all other formulations that contains more than 7% fat, including the two that incorporated 3% SFO (Experiments 1, 2, 3, 5 and 7). This finding (Figure 19b) is similar to what was obtained when applying the TBARS quantification method, thus showing the need to pay attention when incorporating PUFA-rich vegetable oil to avoid excessive lipid oxidation and thus the potential production of off-flavours.

To conclude on lipid oxidation, only the HSB quantification method showed that lipid oxidation increased with time. HCA-based results highlighted that lipid oxidation was more intense for the formulations containing either '21% animal fat' or '7% animal fat plus 3% SFO'. Therefore, care is warranted when using vegetable oil because this type of oil is very sensitive to lipid oxidation due to its high PUFA content.

4.2.5 Protein oxidation

Protein oxidation in DFS samples was quantified by determining (1) carbonyl group content and (2) free thiol group content. In meat samples, higher carbonyl group content means higher protein oxidation but lower free thiol group content.

4.2.5.1 Quantification of carbonyl groups

Figure 20 charts the time-course of carbonyl group content for the first fabrication series as Figure 20a and for the second fabrication series as Figure 20b.

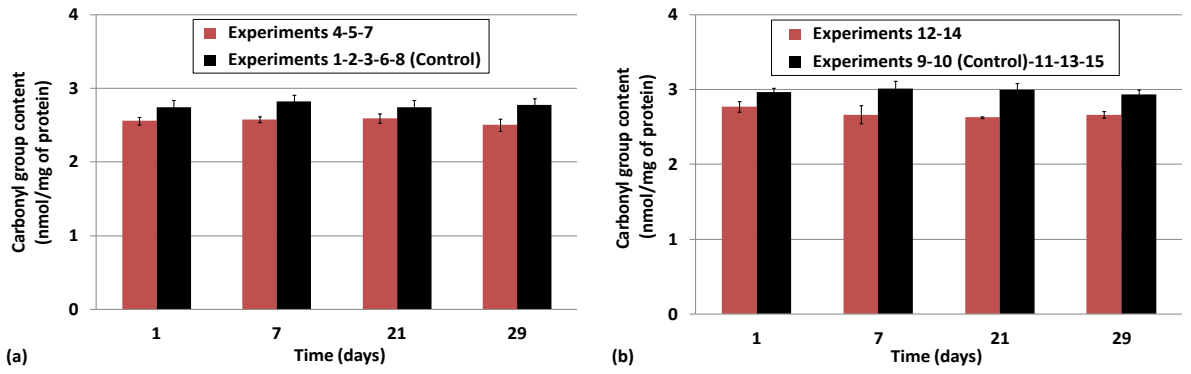


Figure 20. Time-course evolution of protein oxidation quantified by determining carbonyl group content in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw carbonyl group values in order to pool DFS formulations presenting similar patterns.

In both cases, HCA created two classes of formulations as a function of total fat content. Indeed, as shown in Figure 19, whatever the quantification method used, lipid oxidation was greater for the formulations containing most fat: more than 14% animal fat in the first fabrication series (Figure 20a) and 21% animal fat or 7.0% animal fat plus 3% SFO for the second fabrication series (Figure 20b). Since lipid oxidation generates free radicals which,

in turn, can provoke protein oxidation, it is logical to find maximal protein oxidation in the highest-fat formulations, i.e. formulations containing at least 14.7% animal fat (Experiments 1, 2, 3, 6 and 8) in the first fabrication series (Figure 20a) and formulations made with either 21% animal fat or 7% animal fat plus 3% SFO (Experiments 9, 10, 11, 13 and 15) in the second fabrication series (Figure 20b). On the other hand, salt content and type of salt have no visible effect on protein oxidation.

The results obtained on protein oxidation by determining carbonyl group content were identical to those obtained for lipid oxidation by determining TBARS values, with the same formulation classes formed in both cases. On the other hand, surprisingly, carbonyl group content showed no significant change over time. Is this method really suited for assessing and tracking meat protein oxidation?

4.2.5.2 Quantification of free thiol groups

Figure 21 shows the time-course of protein oxidation as determined by free thiol group content. Note that protein oxidation is maximal when free thiol group content is minimal.

For the first fabrication series (Figure 21a), HCA-based results showed three classes of formulation once again classified as a function of their respective fat content. Maximal protein oxidation was observed for the two 21%-animal fat formulations (Experiments 2 and 8). As previously (Figure 20a), minimal protein oxidation was found for the three formulations containing at most 11.6% animal fat. Unlike Figure 20a, an intermediate class constituted of Experiments 1, 3 and 6 was formed from the formulations containing either 14.7% animal fat or 17.9% animal fat (Figure 21a).

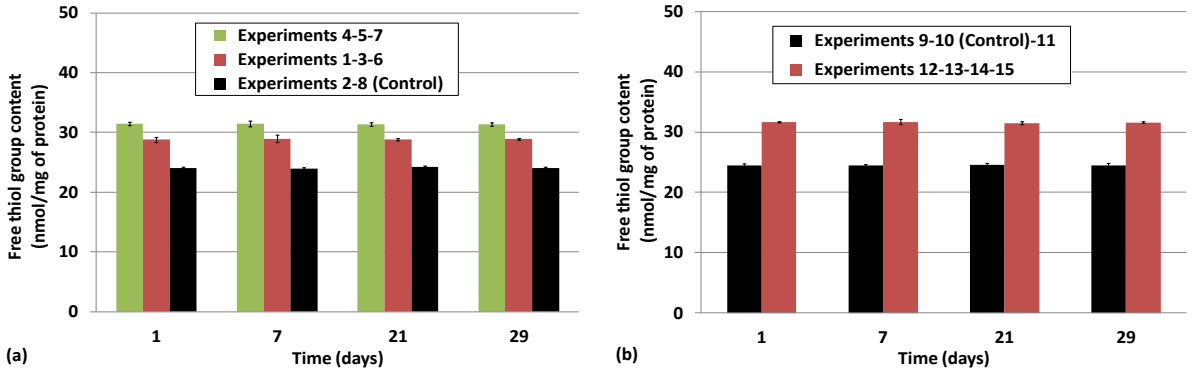


Figure 21. Time-course evolution of protein oxidation quantified by determining free thiol group content in dry-fermented sausages (DFS) for (a) the 8 formulations of the first fabrication series (Experiments 1 to 8) and (b) the 7 formulations of the second fabrication series (Experiments 9 to 15). To facilitate the viewing of results, hierarchical cluster analysis was performed on raw free thiol group values in order to pool DFS formulations presenting similar patterns.

For the second fabrication series, Figure 21b shows that the formulations fell into two classes as a function of their relative animal fat content regardless of the presence or absence of SFO. Unlike Figure 20b, formulations containing SFO (Experiments 13 and 15) are now classed with the 7.0% animal fat formulations and no longer with the 21%-animal fat formulations.

Protein and lipid oxidations are linked by the fact that lipid oxidation produces free radicals that, in turn, drive protein oxidation. Therefore, maximal protein oxidation occurred in high-fat formulations. The adding of SFO seems to also promote protein oxidation. Whatever the experimental quantification method used (carbonyl group content

or free thiol group content), we found no clear change in protein oxidation with time. It would seem that protein oxidation occurs rapidly, maybe directly during the meat batter preparation, and without subsequent intensification.

4.3 DFS Aroma analysis

4.3.1 Identification of odour-active compounds in high-quality DFS

The DHS-GC-MS/8O analyses showed the existence of a broad variety of odours that we collapsed into 8 families: “meaty-animal-dry sausage”, “sulphured-garlic”, “fruity-floral”, “fermented-lactic”, “mouldy-mushroom”, “vegetal”, “empyreumatic” and “plastic-chemical” (Figure 22 and Table 3). The techniques implemented detected 34 odour-active zones with odour intensities greater than 1/5. Among these compounds, 26 were formally identified. Knowing the structure of these volatiles, we could identify their most likely origins. They were mostly substances derived from the degradation of animal tissues during the sausage ripening process or substances deliberately added for flavouring.

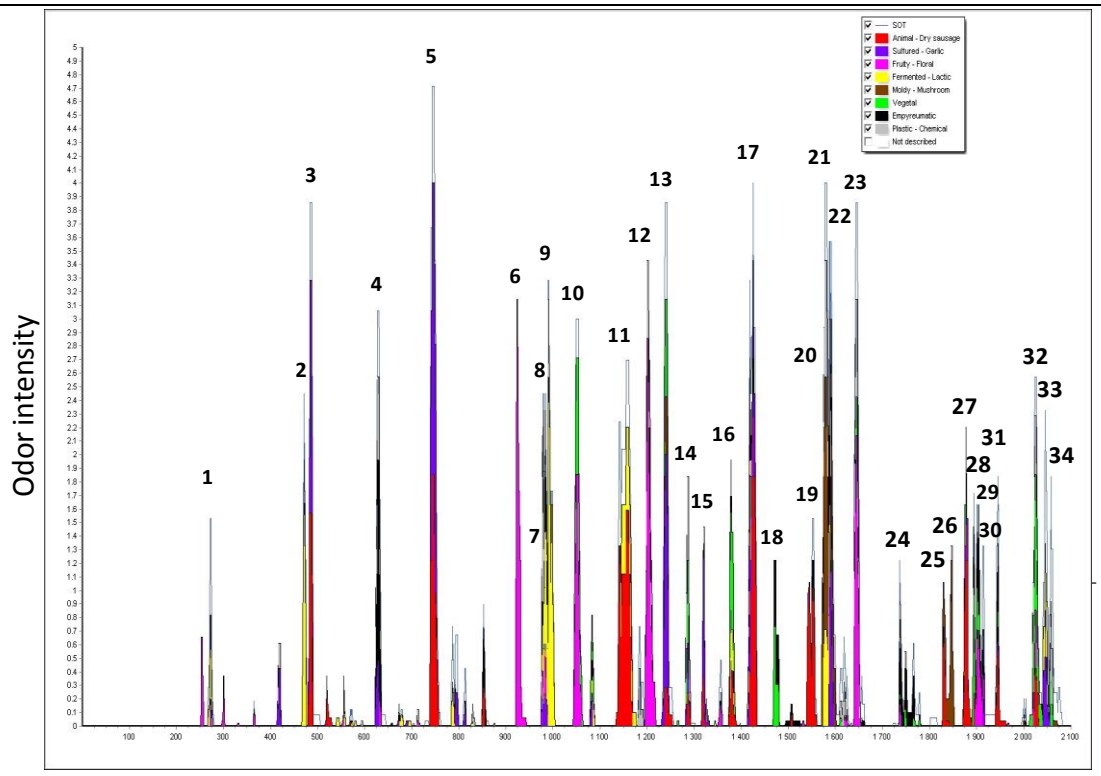


Figure 22. Aromagram obtained by multi-way olfactometry on a mix of high-quality DFS. The peaks correspond to those in Figure 22.

The 15 odour-active volatile substances detected by DHS-GC-MS/8O derived from meat by lipid oxidation (hexanal, butanoic acid, 1-octen-3-ol, 3-octanone, octanal, octanoic acid), amino acid catabolism (3-methylbutanal, 3-methylbutanoic acid, methional, dimethyl trisulphide, benzeneethanol), secondary esterification reactions (2-methylpropanoic acid ethyl ester, 2-methyl butanoic acid ethyl ester, 3-methylbutanoic acid ethyl ester), or partly from the degradation of glycogen (2,3-butanedione).

The 11 odour-active substances derived from flavouring were volatiles originating essentially from garlic (10/11) or pepper (1/11), i.e. alkylsulphides or thiols from garlic and a terpene from pepper. These brought “sulphured-garlic” aromatic notes.

Table 3. List of odour-active compounds identified in a selected mix of traditional DFS. The main sources of the odour-active compounds are indicated as: M = meat, G = garlic, P = pepper, O = other origins.

Chem. Name	(Odour)	Main origin
1 : Unknown1	(cheesy)	O
2 : 2,3-butanedione	(butter)	M
3 : 2-propene-1-thiol	(meaty, garlic)	G
4 : butanal, 3-methyl-	(chocolate)	M
5 : sulfide, allyl methyl	(garlic, dry sausage)	G
6 : propanoic acid, 2-methyl-, ethyl ester	(fruity)	M
7 : toluene	(plastic)	O
8 : thiophene, 3-methyl-	(chemical)	O
9 : butanoic acid	(cheesy, vomit)	M
10 : hexanal	(fruity, green)	M
11 : butanoic acid, 3-methyl-	(animal)	M
12: butanoic acid, 2-methyl-, ethyl ester	(fruity)	M
12: butanoic acid, 3-methyl-, ethyl ester	(fruity)	M
13: allyl sulfide	(garlic, onion, sausage like)	G
14 : alkyl allyl sulphide	(sulphured, gas, dry sausage)	G
15 : unidentified alkyl-thiol	(garlic, gas, sausage)	G
16 : methional	(potatoes)	M
17 : disulfide methyl allyl	(peel of dry cured sausage)	G
18: pinene	(pepper, vegetal)	P
19 : unknown	(animal, peel of dry cured sausage)	O
20 : 1-octen-3-ol	(mushroom)	M
21 : dimethyl trisulfide	(cabbage, gas)	M
22 : 3-octanone	(mushroom, woody)	M
23 : octanal	(citrus, fruity)	M
24 : unknown	(garlic, gas, sausage)	G
25 : unknown	(potatoes)	O
26 : unknown	(dry cured sausage)	G
27 : diallyldisulfide	(garlic, sausage)	G
28 : unidentified terpenoid	(vegetal, woody)	O
29 : benzeneethanol	(floral)	M
30 : unknown	(green, oxidized fat)	O
31 : unknown	(sulphured, meaty)	O
32 : octanoic acid	(rancid, cooked fat	M
33 : Unknown	(sulphured, cheesy)	O
34 : benzene, 1,4-dimethoxy	(vegetal, chemical)	G

The “animal” notes came from the degradation of amino acids such as leucine or isoleucine to 3-methylbutanoic acid. Hydrolysis of triglycerides and/or the oxidative degradation of fatty acids explain the production of butyric and octanoic acid. The “dry-cured sausage”, “dry sausage” and “sausage-like” notes came from the garlic flavouring. In this case, the sniffers associated the “garlic” notes with the odour of sausage as Latin countries typically use garlic flavour DFS, giving it a slight garlic note. The “fruity-floral” notes came from secondary esterification reactions between acids and alcohols of multiple origins, the oxidation of unsaturated fatty acids (hexanal, 1-octen-3-ol, 3-octanone) or amino acids like phenylalanine (benzenethanol). The “potatoe” note came from the oxidative degradation of methionine to methional, which occurs mainly on the sausage surface and is dependent on the fungal flora of the cured products.

To estimate the contribution of the main origins of the aromatic compounds in the DFS aromagram, we calculated the total areas of the odour peaks (i) derived from the biochemical processes of meat maturation during drying, (ii) due to flavouring, and (iii) that were of multiple or indeterminate origins (Figure 23). This simple calculation clearly shows that the DFS aroma requires a very strong contribution from substances derived from the degradation of certain amino acids, oxidation of fatty acids, and secondary esterification reactions, given that 50% of the intensity of the odours detected by olfactometry came from the transformation of the meat matrix during drying. Flavouring, including garlic, also plays a key role in the construction of the final aromatic properties of DFS. This key role can be explained by both the low detection threshold of sulphur-containing compounds and their individual flavouring properties. These compounds have intense "garlic" notes as well as "dry-sausage" notes that give the final note to the overall DFS aroma, very probably by masking much of the other odour-active compounds during tasting or direct sniffing of sausages. The aromatic role of pepper proved much more limited, and probably influenced taste more than aroma; we were unable to evaluate this point by chromatography-olfactometry.

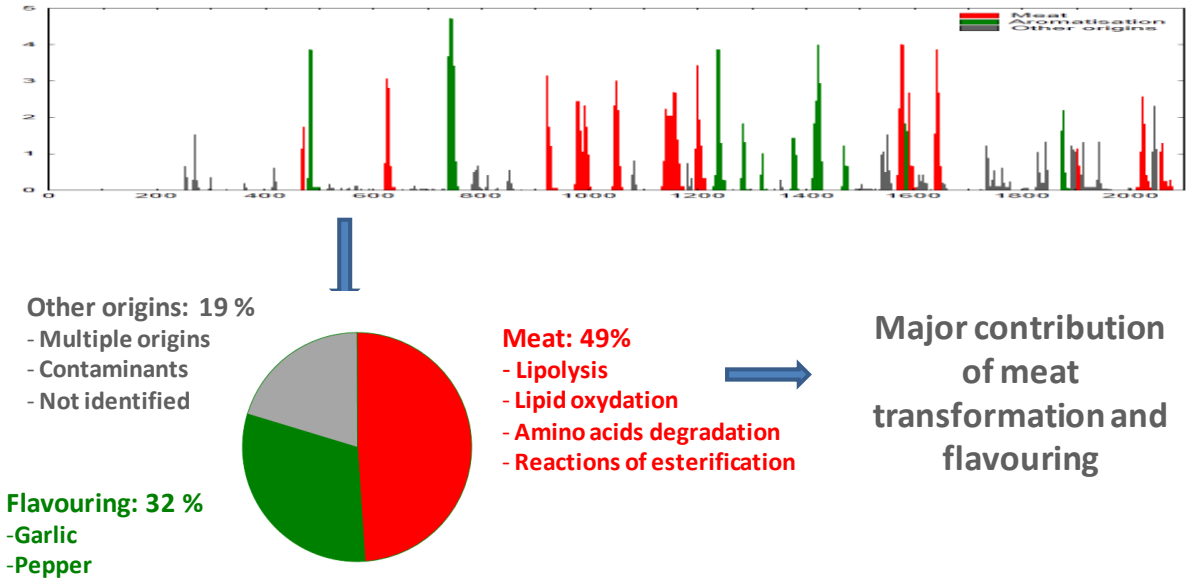


Figure 23. Aromagram obtained by multiway olfactometry on a mix of high-quality DFS. The peak numbers correspond to those in Table 1.

4.3.2 Analysis of the volatile fraction of low-fat low-salt flavoured DFS

The reclassification of formulations from the volatile markers in DFS shows that the most striking effects were produced by reducing fat (Figure 24), i.e. Class C1: low-fat and Class C2: 21% fat. The dried sausages containing 21% fat were thus those that had desorbed most compounds derived from lipid oxidation (aldehydes, methylketones and alcohols).

For example, Figure 25 shows that the desorption of 1-octen-3-ol, derived from the degradation of unsaturated fatty acids, was nearly five times stronger in the non-low-fat products, yet the strong mushroom odour of this compound was never described by sniffers in sensory evaluations. In contrast, lipid and amino acid catabolism was not significantly affected by fat and salt reductions or substitutions.

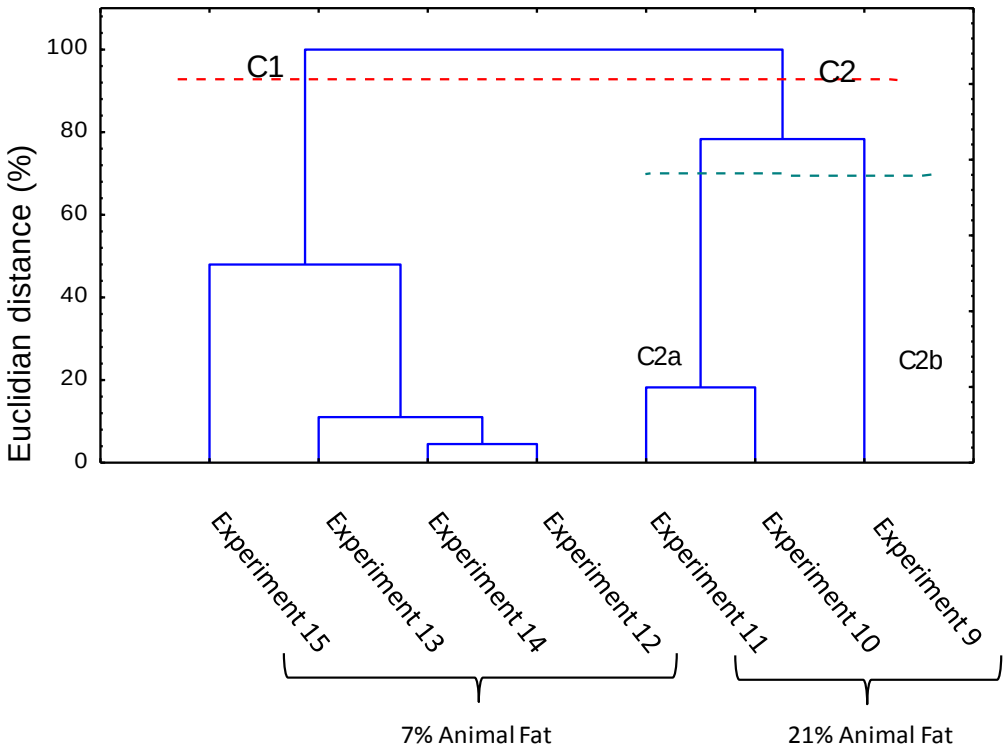


Figure 24. Classification of the formulations obtained from the analysis of their volatile fraction analyzed by GC-MS. Computations were performed from markers of carbohydrate catabolism (2,3-butanedione), lipid oxidation (hexanal, heptanal, octanal, nonanal, 2-heptanone, 2-octanone, 2-nonanone, 1-octen-3-ol, 1-octen-3-one), amino acid catabolism (butanal, 3-methyl-, butanal, 2-methyl-, butanoic acid, 3-methyl-, butanoic acid, 2-methyl-, ethanol, 2-phenyl-) and aromatization (2-propene-1-thiol, 1-propene, 3-methylthio or sulfide, allyl methyl, disulfide, methyl allyl, alpha-pinene). A red dotted line separates class C1 from class C2. A green dotted line separates class C2a from C2b.

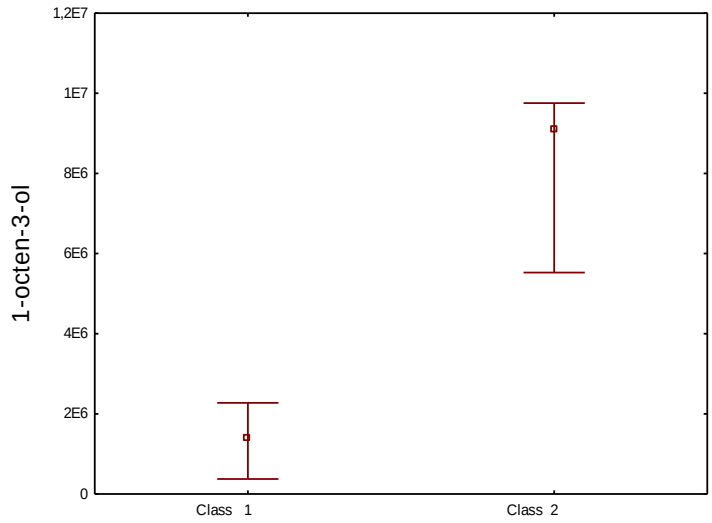


Figure 25. Desorption of 1-octen-3-ol, derived from the degradation of unsaturated fatty acids, analyzed by dynamic headspace sampling coupled to gas chromatography-mass spectrometry. Formulations of Class 1 (equivalent to Class C1 of Figure 24) are low-fat content while formulations of Class 2 (equivalent to Class C2 of Figure 24) are non-low-fat content (21% animal fat).

Class C2 of Figure 24, which groups fat-rich formulations, contains two subclasses (C2a and C2b) which separate flavoured formulations (C2a) from non-flavoured formulations (C2b). Class C2a shows that disruptions to the volatile fraction induced solely by the addition of KCl are reduced because they are close in the tree diagram. In Class C1, the disruptions induced by substituting fat by SFO and NaCl by KCl (Class C1) are difficult to interpret, especially as the effect of the substitutions was probably masked by the flavouring (Figure 24).

4.4 Evaluation of DFS quality and consumer acceptance

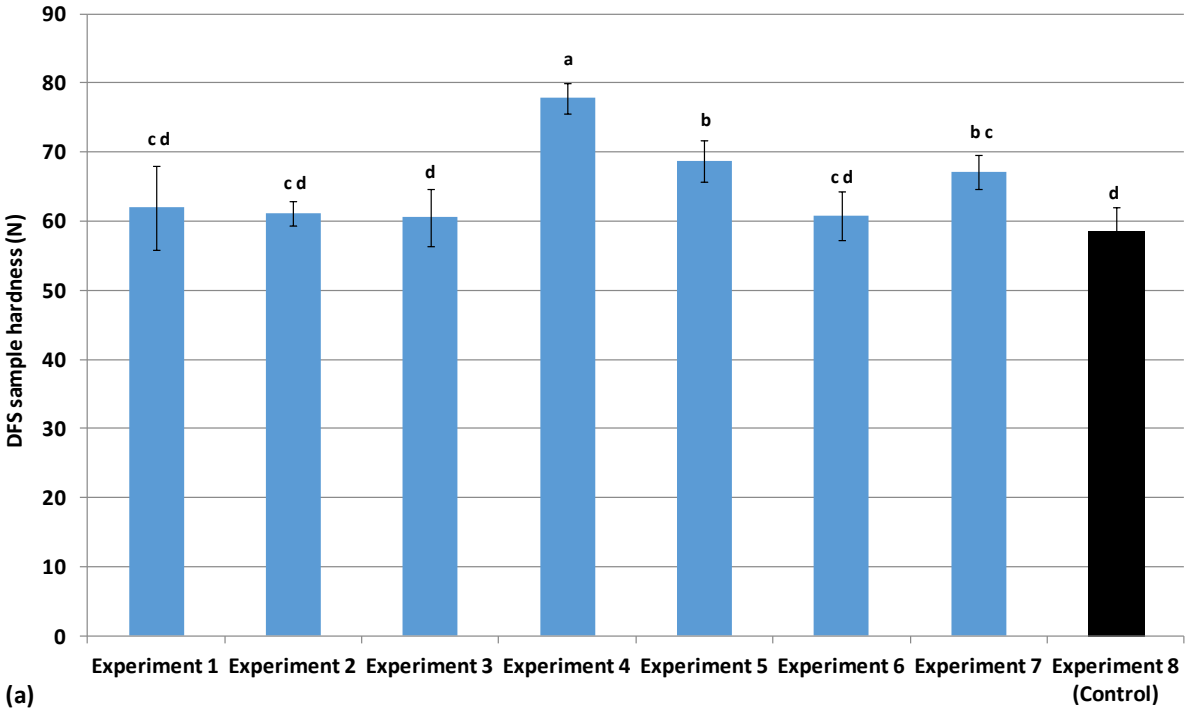
4.4.1 Texture profile analysis

4.4.1.1 First series of DFS fabrication

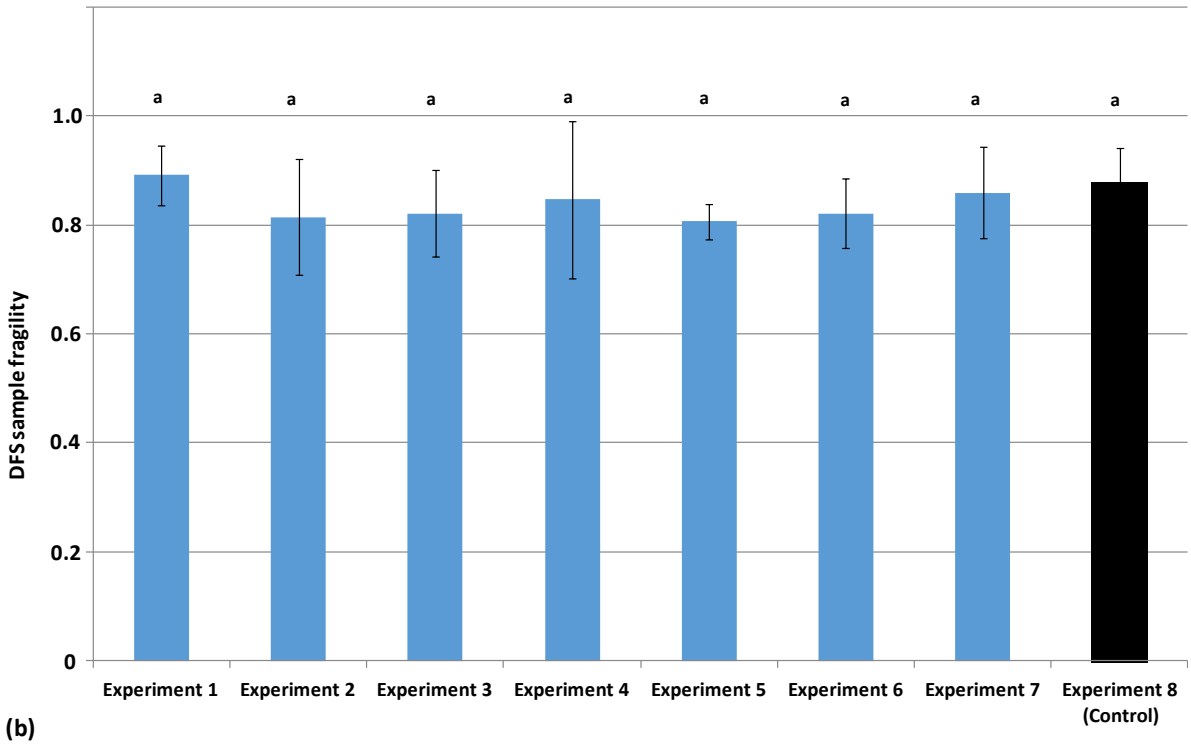
For the first series of DFS fabrication reported in Table 1, Figure 26 shows the values of the four textural parameters (hardness, fragility, cohesiveness and elasticity) measured by TPA-testing samples extracted from 29-day-old DFS. Analysis of Figure 26 informs on how NaCl and animal fat contents affect these textural parameters.

Global analysis of figure 26 indicates that:

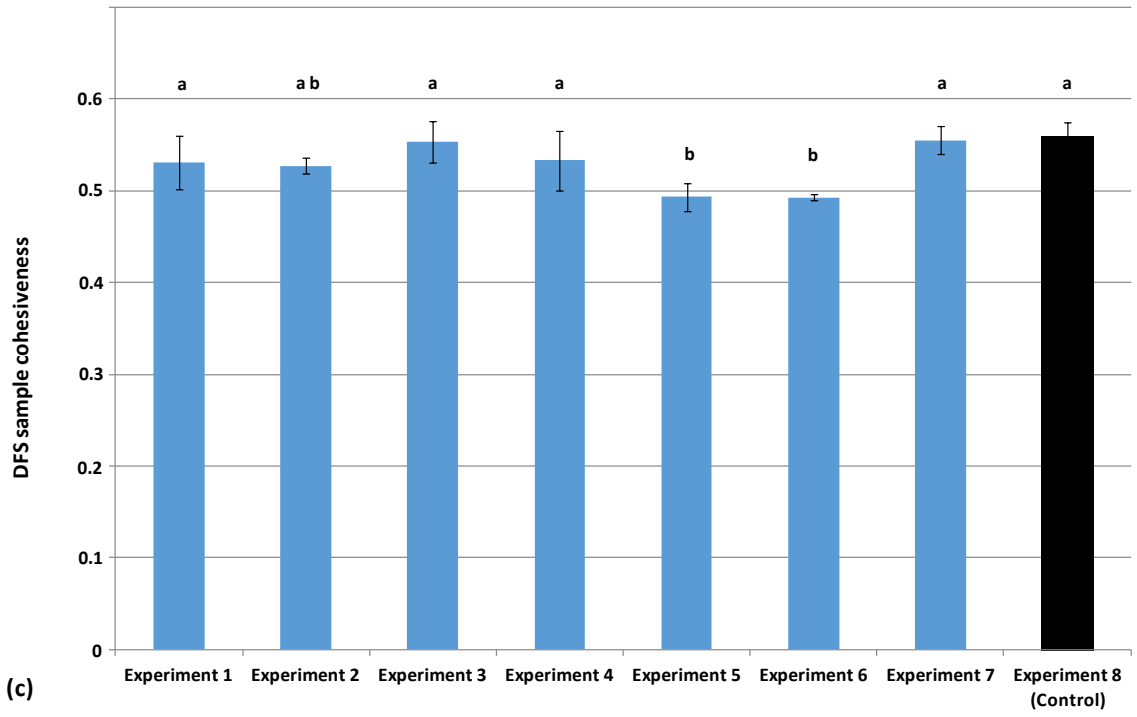
- (1) Regarding hardness (Figure 26a), the highest values were obtained for Experiments 4, 5 and 7 of Table 1, i.e. the 3 low-fat fabrications (8.4% and 11.6% animal fat content, respectively), thus highlighting a highly significant effect of animal fat content on final DFS texture ($p < 0.001$). These high values are probably due to higher water loss of the DFS during drying, as visible in Figure 10a. On the other hand, statistical analysis did not find any significant effect of NaCl content in the range 2.0%–2.8% on the textural properties of DFS.
- (2) Regarding fragility (Figure 26b), we found no significant difference between the 8 experiments on this textural parameter, despite the fact that fragility is linked to product hardness. Remember that fragility is calculated from the ratio of peak force of the second compression to the peak force of the first compression that gives sample hardness.
- (3) Regarding cohesiveness (Figure 26c), the lowest values were obtained for Experiments 5 and 6, i.e. the two formulations containing only 2% NaCl, thus highlighting a highly significant effect of salt content ($p < 0.001$) on final product cohesiveness. These low cohesiveness values probably result from more intense proteolysis, as shown in Figure 15a. On the other hand, we found no significant effect of animal fat content on product cohesiveness.
- (4) Regarding elasticity (Figure 26d), statistical analysis indicated that animal fat content has a highly significant effect ($p < 0.001$) on elasticity value, unlike salt content (although the effect of salt on DFS elasticity was at the limit of significance, $p = 0.07$). Figure 26d shows that the highest elasticity values were measured for Experiments 4 (the lowest-fat formulation) and 7 (a low-fat but high-salt formulation). We found little difference elasticity values for the other 6 formulations.


DFS sample hardness (N)
90
80
70
60
50
40
30
20
10
0
c d
c d
d
a
b
c d
b c
d
Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Experiment 6
Experiment 7
Experiment 8 (Control)

(a)


DFS sample fragility
1.0
0.8
0.6
0.4
0.2
0
a
a
a
a
a
a
a
a
Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Experiment 6
Experiment 7
Experiment 8 (Control)

(b)



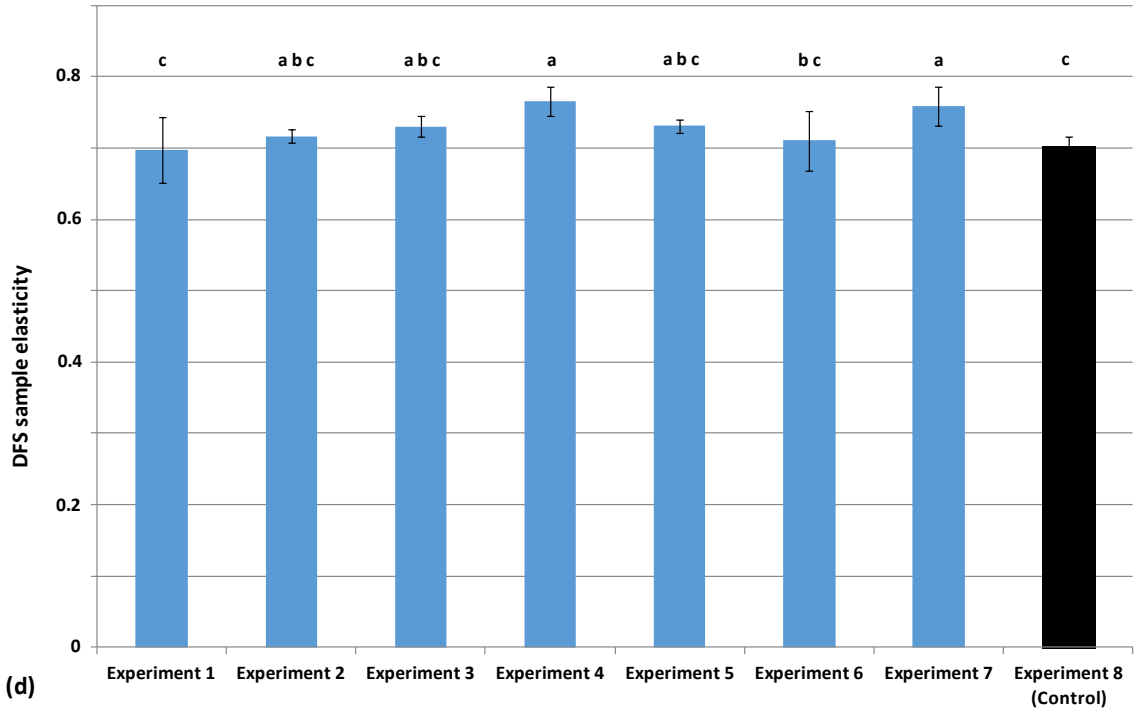


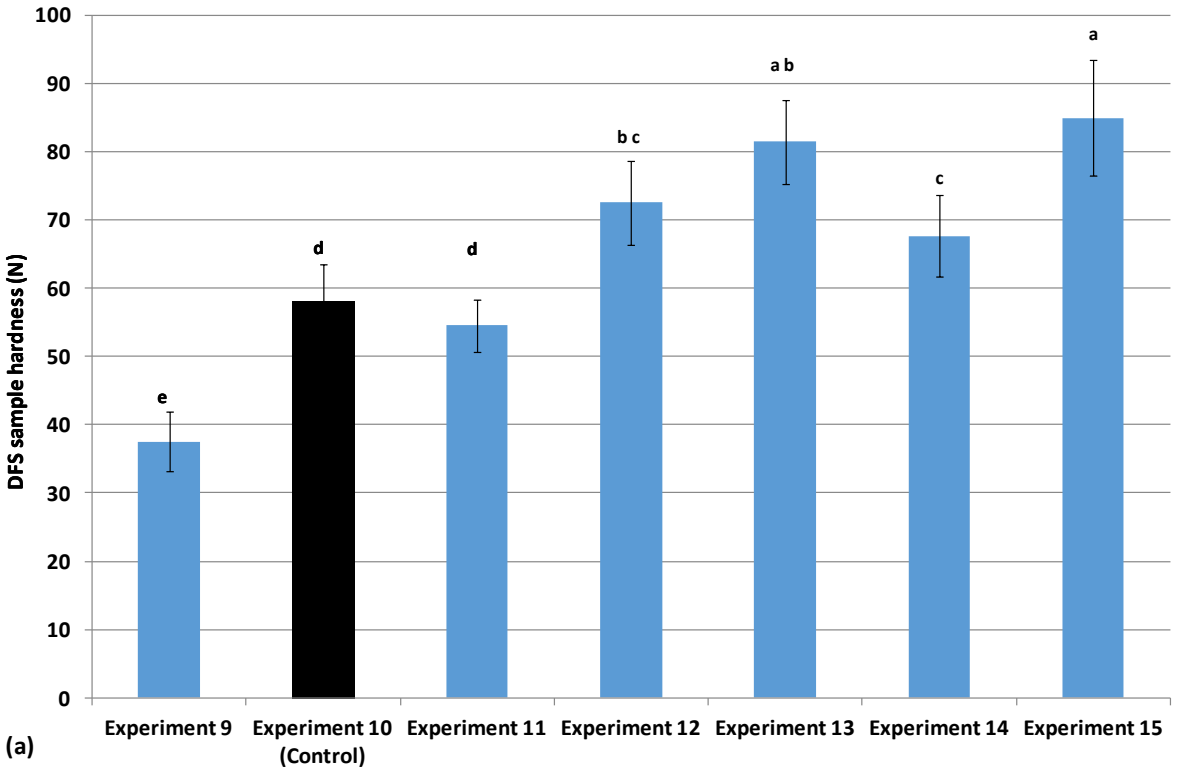
Figure 26. Values of four textural parameters, i.e. (a) hardness, (b) fragility, (c) cohesiveness and (d) elasticity, measured by TPA-testing samples extracted from 29-day-old DFS for the first of fabrication series of Table 1. Values are means ± SD of 6 independent determinations (n=6). Values not bearing common superscripts differ significantly ($p < 0.05$).

4.4.1.2 Second series of DFS fabrication

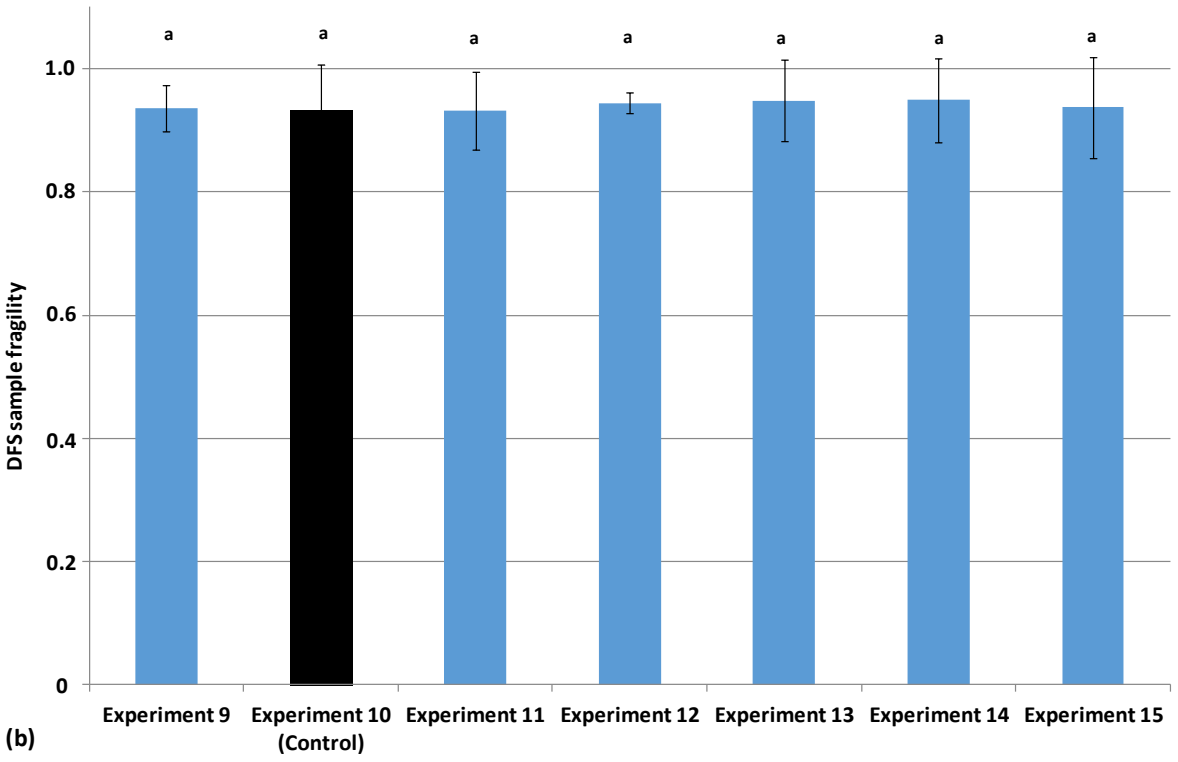
For the second series of DFS fabrication reported in Table 2, Figure 27 shows the values of the four textural parameters (hardness, fragility, cohesiveness and elasticity) measured by TPA-testing samples extracted from 29-day-old DFS. Analysis of Figure 27 informs on how direct reduction of NaCl, direct reduction of animal fat content, partial substitution of NaCl by KCl, added vegetable oil and flavouring can affect these textural parameters.

Global analysis of figure 27 shows that:

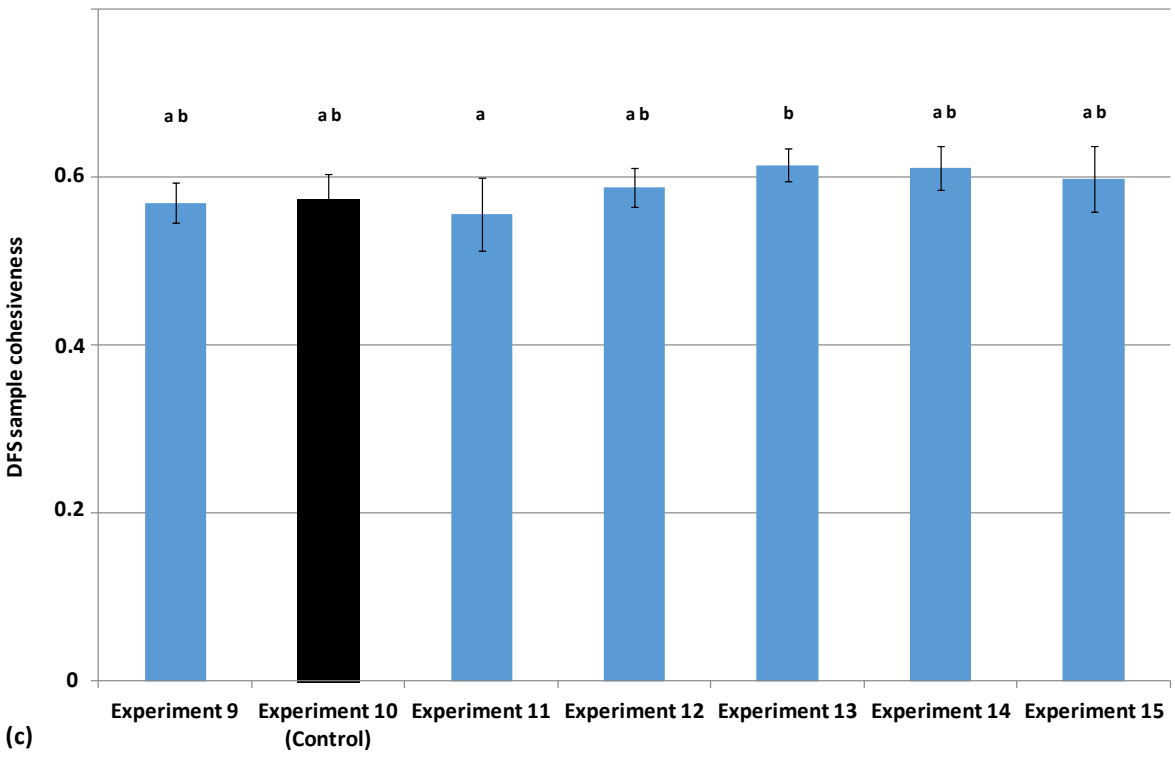
- (1) Regarding hardness (Figure 27a), the highest values were obtained for the 7%-animal fat formulations (Experiments 12 to 15) compared to the 21%-animal fat formulations (Experiments 9 to 11), thus again highlighting a highly significant effect of fat content on final DFS texture ($p < 0.001$). Also, adding vegetable oil (Experiments 13 and 15) clearly modified DFS texture, making them harder. Comparing Experiment 11 *vs* Experiment 10 and Experiment 14 *vs* Experiment 12 highlights a very limited impact of using KCl as an NaCl substitute on DFS texture. On the other hand, not adding flavouring (garlic and pepper) was detrimental to final DFS texture, probably due to under-acidification during the fermentation stage that led to a visibly poor sliceability. Finally, statistical analysis found no significant effect of NaCl or KCl content on the textural properties of DFS.
- (2) Regarding fragility (Figure 27b), like for the first fabrication series, the 7 experiments did not differ significantly on this textural parameter, despite it being linked to product hardness.
- (3) Regarding cohesiveness (Figure 27c), slightly lower values were obtained for the 21%-animal fat formulations (Experiments 9 to 11) than the 7%-animal fat formulations (Experiments 12 to 15). Nevertheless, statistical analysis found a significant effect of fat content ($p < 0.01$) but no significant effect of added vegetable oil, salt content or type of salt on final product cohesiveness.
- (4) Regarding elasticity (Figure 27d), analysis found the same patterns as for cohesiveness, i.e. slightly lower values for the 21%-animal fat formulations (Experiments 9 to 11) than the 7%-animal fat formulations (Experiments 12 to 15), but with statistical analysis indicating a highly significant effect ($p < 0.001$) of the animal fat content on elasticity value. There was no visible or statistically significant effect of salt content, type of salt or adding SFO on DFS elasticity.


DFS sample hardness (N)
100
90
80
70
60
50
40
30
20
10
0
e
d
d
b c
a b
c
a
Experiment 9
Experiment 10 (Control)
Experiment 11
Experiment 12
Experiment 13
Experiment 14
Experiment 15

(a)


DFS sample fragility
1.0
0.8
0.6
0.4
0.2
0
a
a
a
a
a
a
a
Experiment 9
Experiment 10 (Control)
Experiment 11
Experiment 12
Experiment 13
Experiment 14
Experiment 15

(b)



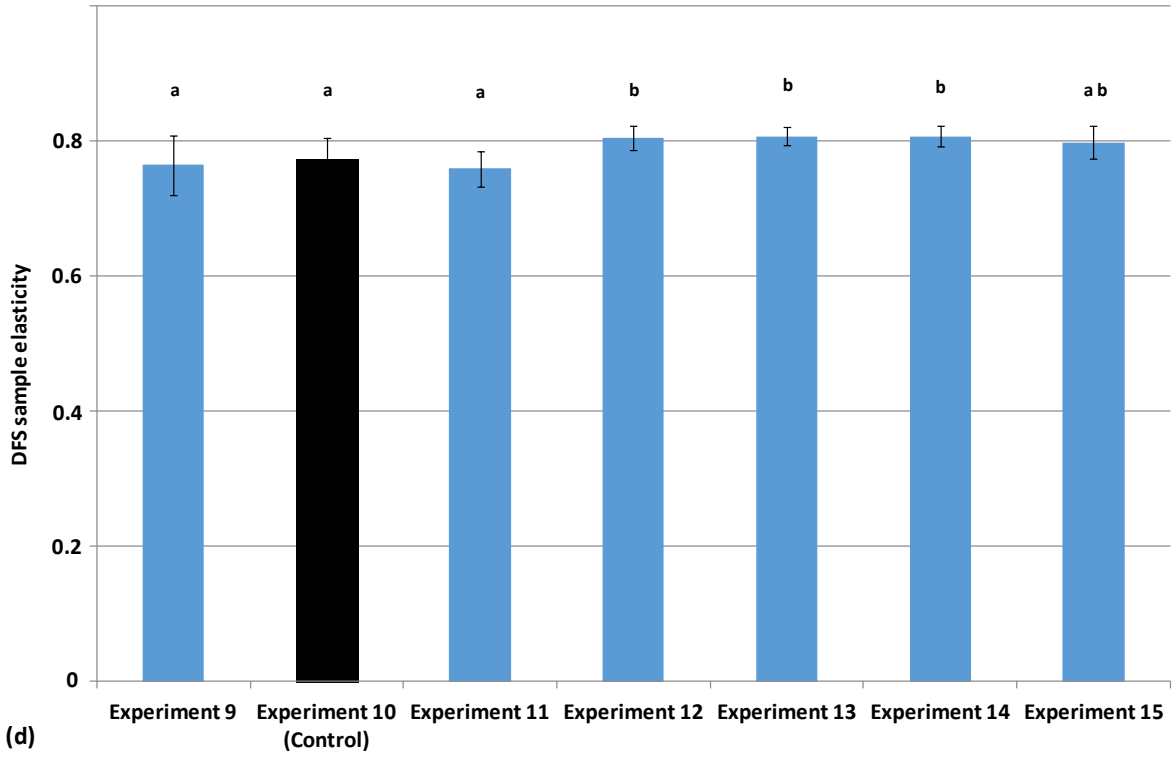


Figure 27. Values of four textural parameters, i.e. (a) hardness, (b) fragility, (c) cohesiveness and (d) elasticity, measured by TPA-testing samples extracted from 29-day-old dry-fermented sausages (DFS) for the second of fabrication series of Table 2. Values are means ± SD of 6 independent determinations (n=6). Values not bearing common superscripts differ significantly ($p < 0.05$).

4.4.2 Sensory acceptability of low-fat and low-salt formulations

Classification of the assessors according to the four acceptability criteria revealed three types of behaviour (Figure 28). The assessors in Group 1 (n = 6/29) scored the experimental products less highly than the assessors in Groups 2 and 3 who formed a large majority (n = 23/29). The lower scores given by Group 1 can be explained by the fact that these were consumers of high-quality crafted cured products, whereas the ADIV products were ordinary-quality sausages. With no statistical interaction between assessor and formulation over the four criteria evaluated, the differences in acceptability between the three groups of assessors were mainly ascribed to scoring the DFS with ranging severity according to assessors. The absence of interactions between assessors also enabled us to consider the assessors as forming a single group with homogenous behaviour, and thus to analyze the average responses of the panel of assessors to study the effects of the formulations on the acceptability scores (Figure 29).

The acceptability of the sausages was judged mediocre only for the non-flavoured formulation "NaCl 2.8% + fat 21%, Experiment 9" (scores for appearance, texture, taste and aroma between 3.7 and 4.7/10) and for the formulation "NaCl 2% + KCl 0.8% + fat 7% + Flavouring, Experiment 14" which scored low on aroma (aroma defects, metallic taste, etc.). For all the other formulations of Table 2, acceptability scores for most of the criteria lay between 5.5 and 6.5/10. These acceptability scores were slightly higher than average, as the ADIV products were ordinary-quality DFS in synthetic casings of standard size, which pre-positioned the sausages in a mid-quality range. The products that scored highest on all the criteria corresponded to formulations "NaCl 2% + KCl 0.8% + fat 7% + SFO 3% + Flavouring, Experiment 15" and "NaCl 2.8% + fat 7% + SFO 3% + Flavouring, Experiment 13", with slightly higher acceptability scores than for the reference formulation "NaCl 2.8% + fat 21% + Flavouring, Experiment 10" (Figure 29).

There are several reasons for the large gap in acceptability of the non-flavoured reference formulation "NaCl 2.8% + fat 21%, Experiment 9". This formulation presented a less refined appearance (the ingredients giving it a darker, more mat colour) than the other formulations due to the absence of flavouring additives, which naturally contain fermentation activators that boost the activity of microbial starter cultures. The absence of flavouring thus limited the acidification process, with a strong impact on final DFS texture and aroma. Finally, flavouring enhanced acceptability by more than one point irrespective of formulation.

If we refer to the comments made by the assessors during the tasting session (Table 4):

- All the DFS containing 21% fat (Experiments 9 to 11) had higher "animal, pork, fatty" aroma scores and higher "fatty, buttery" scores than the low-fat low-salt products. In the absence of flavouring (Experiment 9), these products were often described as "dry cured ham" and "not flavoured", which certainly tended to diminish their acceptability in a DFS tasting context. In addition, the presence of fat limited drying and caused problems of poor cohesion between the fat and the lean meat components, which the assessors readily discerned.
- For most of the flavoured low-fat low-salt products, the similar acceptability scores on the various criteria can be explained by their pleasant texture, good cohesion despite an appearance found too closely blended, firm and somewhat rubbery. Despite marked reductions in NaCl content, the flavour of the sausages was always found salty enough, or even too salty, even when NaCl was replaced by KCl. In the case of salt reduction, the flavouring probably acted as a saltiness enhancer, as the assessors made very frequently associated items such as "too salty and spicy".

- In the case of the formulation "NaCl 2% + KCl 0.8% + fat 7% + Flavouring, Experiment 14", "vegetal" aromatic notes and aroma defects or an "insipid" aroma were reported. For this formulation, it seems difficult to incriminate KCl, as it was used in two other formulations of Table 2 (Experiments 11 and 15) without generating any detrimental defect.

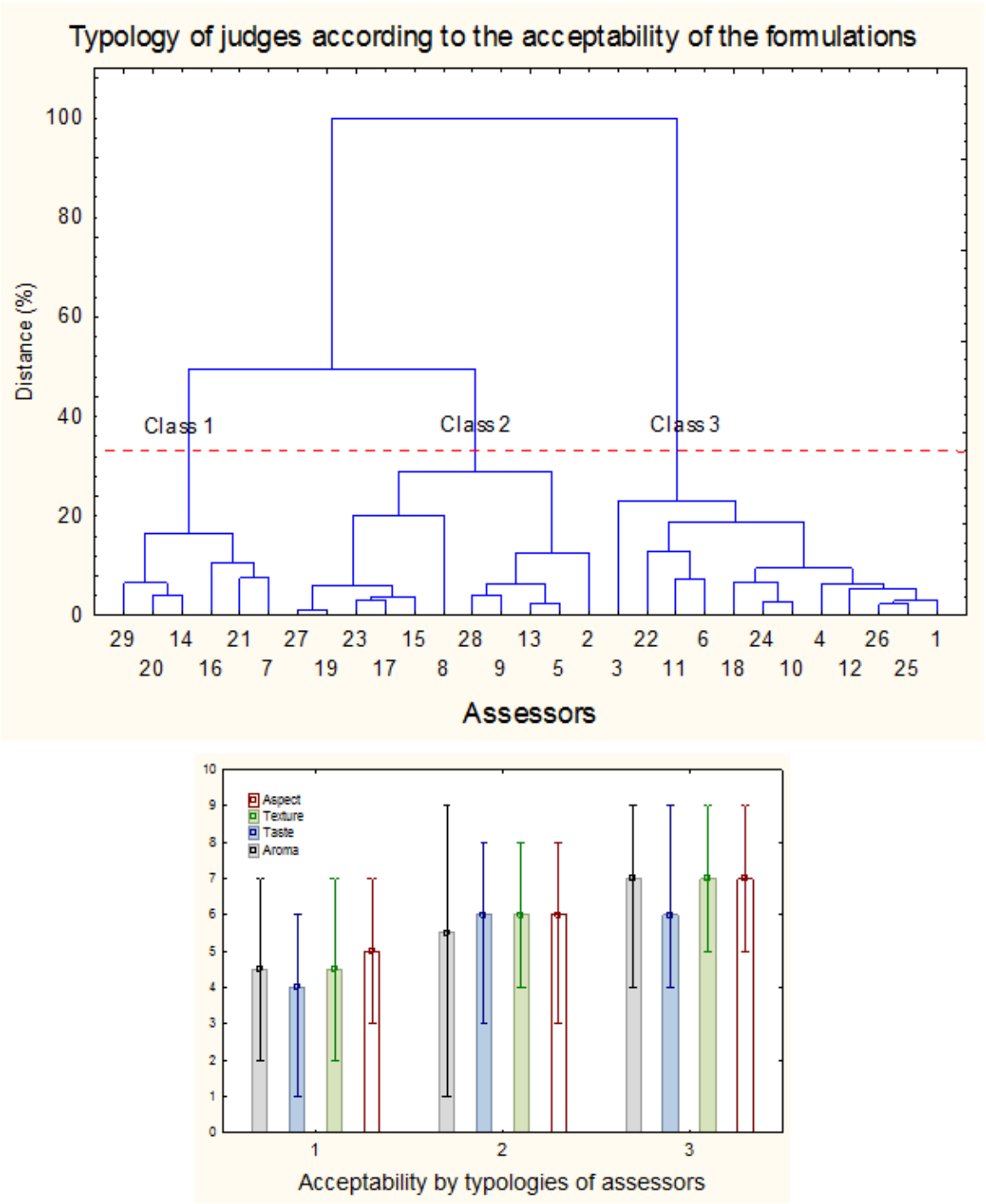


Figure 28. Main classes of judges obtained by hierarchical cluster analysis. Computations were performed from the scores on acceptability, appearance, texture, taste and aroma. At the chosen threshold (red dashed line), 3 classes were identified. Acceptability ratings for different criteria are presented as bar charts (median, quartiles).

Table 4. Key comments made by the 29 assessors on the organoleptic characteristics of the different formulations of DFS corresponding to the second fabrication series as reported in Table 2. F means Flavouring.

FORMULATION	TEXTURE	TASTE	AROMA
Experiment 9: NaCl 2.8% + Fat 21%	Not dry enough, too fat, poor cohesion, soft.	Fat flavour, acid, bland, too salted.	Low aroma, without aroma, dry-cured ham aroma, not flavoured, fatty, butter.
Experiment 10 (Control): NaCl 2.8% + Fat 21% + F	Irregular texture, bad hash, too fat, poor cohesion, soft.	Fat flavour, too salted.	Low aroma, animal, pork aroma, nutty, butter.
Experiment 11: NaCl 2.0% + KCl 0.8% + Fat 21% + F	Too mixed, rubbery, poor cohesion.	Salted and spicy.	Pork flavour, flavoured, pleasant.
Experiment 12: NaCl 2.8% + Fat 7% + F	Too mixed, not firm texture, dry- cured ham texture, pleasant texture.	Good salty and piquant (pepper).	Too spicy, limited sausage aroma.
Experiment 13: NaCl 2.8% + Fat 7% + SFO 3% + F	Mixed, firm but pleasant, good cohesion, rubbery, dry.	Too salty.	Good aroma, spicy, pleasant.
Experiment 14: NaCl 2.0% + KCl 0.8% + Fat 7% + F	Pleasant texture, not too fat.	Too salty and piquant.	Low aroma of sausage, vegetal aroma, off-flavours.
Experiment 15: NaCl 2.0% + KCl 0.8% + Fat 7% + SFO 3% + F	Bad texture, rubbery.	Too salty and piquant.	Low aroma of sausage, bland

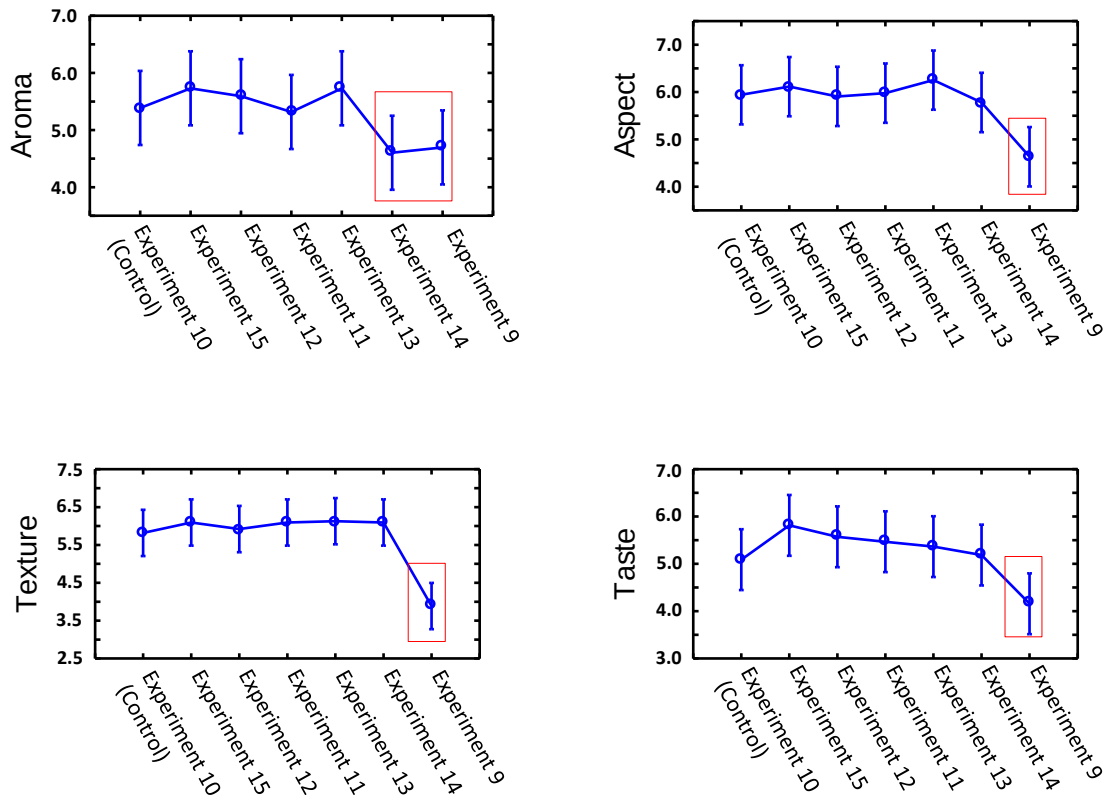


Figure 29. Acceptability of aspect: texture, aroma, and taste of the dried sausages. Formulations of sausage outlined in red are significantly different ($p < 0.05$ for aspect, aroma, and taste or $p < 0.01$ for texture) to the others. Means and confidence interval are shown.

5. Conclusions

On account of the main findings reported in Deliverable 2.1 and in accordance with the main results of the literature, the concrete objective set for Task 2.3 was to run two series of DFS manufacture to bring accurate data on the following three points:

- The potential application of new technologies in DFS production (adding KCl as a substitute for NaCl, adding vegetable oil...) to lower SFA and sodium content,
- The impact of fat and salt reduction on water and salt transfers and the formation of odour and flavour compounds,
- The product quality and consumer acceptability of low-sodium and low-fat DFS.

DFS drying globally leads to a reduction in in-DFS water content due to water evaporation from the DFS surface and, in turn, to a fat and salt concentration that increases fat and salt content, respectively. For the two fabrication series, the respective proportions of lean meat and fat logically influence the in-DFS water content values. For an identical drying process, the highest water content values expressed in percentages are obtained for the low-fat products. The measured salt content values (NaCl and KCl) at Day 1 were still higher than the intended values, probably as a result of the natural presence of sodium, chloride and potassium ions in lean pork meat and very probably as a result of real difficulties in perfectly adjusting the amount of salt added during the meat batter preparation as a function of the respective proportions of lean pork meat and fat. However, the formulations with the higher intended salt content values nevertheless contained more salt (NaCl and KCl) than the others, once the meat batters were prepared, thus *a priori* lowering the impact of this observed discrepancy on the results subsequently obtained. In a similar way to salt content, there were discrepancies in total lipid content reaching 2% at most, again underlining the real difficulty in perfectly adjusting the amount of added fat during meat batter preparation. However fortunately, the formulations with the higher intended fat content values really contained more fat than the others, once the meat batters were prepared.

Time-course pattern of physicochemical parameters was also measured for DFS of the two fabrication series, namely DFS weight loss, mean in-DFS a_w and mean in-DFS pH values. Concerning DFS water loss, the present results showed a strong impact of animal fat content whatever the fabrication series, with about 8% variation in DFS water loss according to the formulations, and a moderate effect of salt content, exclusively for the first DFS fabrication series. Concerning DFS water loss, whatever the fabrication series, the results logically showed a strong impact of salt content, an impact of type of salt (NaCl or KCl), and also an effect of fat content. From a water activity perspective, reducing fat content in DFS provokes the same increase on a_w as reducing salt content. So, binary reductions in DFS fat and salt content may prove detrimental from a safety standpoint if the products are not sufficiently dried. Concerning in-DFS pH value, measurements showed normal time-course of pH value, with two distinct phases: strong acidification during the first week of process followed by a progressive increase in pH value, except in the non-flavoured formulation. This therefore highlights a strong impact of flavouring on time-course of pH values, an impact of salt content, and a moderate effect of fat content. No discernible effect of type of salt (NaCl or KCl) was found.

The main conclusion from the MBRA drying trials is that surface microbial flora growth did not modify water transfers inside and at the surface of the product. This means that the water transfers (evaporated water flux, a_w and weight loss) in the dried products investigated here were directly induced by the drying conditions applied. Therefore, we built a sorption isotherm making it possible to calculate a_w value as a function of water content, NaCl content and fat content anywhere in a DFS.

Time-course of the biochemical parameters was also measured for DFS in the two fabrication series, namely proteolysis, lipolysis, lactic acid content, lipid oxidation and protein oxidation. The results confirmed that proteolysis in DFS was mainly governed by their salt content. Reducing the salt content increases proteolysis, which can be detrimental for the final texture of the end-products. Concerning lipolysis, the data seems to suggest that the intensity of this biochemical phenomenon is mainly dependent on fat content, but further quantification of lipolysis is needed, maybe using another experimental method than determination of acid value, to definitively conclude on the effect of reducing salt and fat content on DFS lipolysis. Concerning lactic acid content, acid production rates fit perfectly with pH time-course values. Concerning lipid oxidation, only the HSB quantification method showed that lipid oxidation increased with time. HCA-based results highlighted that lipid oxidation was more intense for the formulations containing either '21% animal fat' or '7% animal fat plus 3% SFO'. Therefore, care is warranted when using vegetable oil because this type of oil is very sensitive to lipid oxidation due to its high PUFA content. Protein and lipid oxidations are linked by the fact that lipid oxidation produces free radicals that, in turn, drive protein oxidation. Therefore, maximal protein oxidation occurred in high-fat formulations. The adding of SFO seems to also promote protein oxidation. Moreover, we found no clear change in protein oxidation with time. It would seem that protein oxidation occurs rapidly, maybe directly during the meat batter preparation, and without subsequent intensification.

Analysis of DFS aroma indicates that the two main origins of the aroma of dry sausages can be assigned to (i) odour-active compounds forming during the degradation of animal tissues over the course of the fermentation and drying processes, and (ii) flavouring with natural substances. Given that the manufacturing recipes used by ADIV are widely recognized as industry-standard practice and that it is extremely difficult to steer aroma in salt-cured products by acting solely on the fermentation processes (via seeding flora or product refining, etc.), we elected to enhance the aroma of low-salt low-fat sausages by adding flavouring with natural substances. The results of gas-phase chromatography coupled with olfactometry prompted us to preferentially introduce odour-active compounds with "meaty" or "dry-cured" notes, which the assessors considered typical of cured products. The simplest way to do this was to flavour our experimental low-salt low-fat products mainly with a garlic-based extract. ADIV thus chose a powdered dried garlic extract that was easier to add and blend into the mixture. Black pepper was also added in the formulations. These two ingredients are additives already used separately or jointly in French manufacture of traditional products, and so their use in low-salt low-fat products should not surprise consumers. Moreover, the profiling of odour-active compounds identified in the DFS shows that the production of compounds formed by lipid oxidation depends largely on in-sausage fat level. Replacing NaCl by KCl proved to have limited effect on volatile biochemical markers.

The results clearly show that salt and fat contents may be greatly reduced with no adverse effect on the final texture properties and acceptability of DFS: most of the low-fat low-salt flavoured products presented practically the same texture and acceptability as full-fat full-salt reference DFS (control), even though their organoleptic characteristics were different. The role of flavouring proved very important, as it acted not only through the introduction of aromatic substances that enhance the acceptability of the aroma but also by activating fermentation processes that further shape texture acceptability. Flavouring, mainly with garlic, is one possible solution that we can advocate, since garlic has a long history use in French dry-cured meat products. Ultimately, various flavouring solutions will probably have to be implemented according to consumer tastes and eating habits in the countries or regions concerned in order to optimize the acceptability of new low-fat low-salt products. This is something that will be tested under WP6 in the case of Spanish chorizo, via a collaboration with ADIV and the industry manufacturer Boadas 1880 S.A.

6. References

Armenteros, M., Aristoy, M.C., Barat, J.M. & Toldrá, F. (2009). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry*, 117, 627-633.

Benedini, R., Parolari, G., Toscani, T. & Virgili, R. (2012). Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Science*, 90, 431-437.

Berdagué, J.L. & Tournayre, P. (2002). The "Video-Sniff" method, a new approach for the "Vocabulary-Intensity-Duration" study of "Elementary Odours" perceived by gas-chromatography-olfaction. *In Tec & Doc Editors (Ed.), Flavour Research at the Dawn of the Twenty-First Century (pp. 514-517)*. Paris, France.

Berdagué, J.L. & Tournayre, P. (2005). Gas chromatography-olfaction analysis device and method. *Patent WO 2005/001470 A2*, France: Institut National de la Recherche Agronomique.

Berdagué, J.L., Tournayre, P. & Cambou, S. (2007). Novel multi-gas chromatography-olfactometry device and software for the identification of odour-active compounds. *Journal of Chromatography A*, 1146(1), 85-92.

Beriain, M.J., Gomez, I., Petri, E., Insausti, K. & Sarries, M.V. (2011). The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Science*, 88, 189-197.

Bourne, M.C. (2002). Principles of objective texture measurement. *In M. C. Bourne (Ed.), Food texture and viscosity: Concept and measurement (pp. 107–188)*. San Diego, USA.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. Meat Science, 74, 188-196.

Doehlert, D.H. (1970). Uniform shell designs. *Applied Statistics*, 19, 231-239.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues *The Journal of Biological Chemistry*, 226(1), 497-509.

Gou, P., Guerrero, L., Gelabert, J. & Arnau, J. (1996). Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry-cured pork loin. *Meat Science*, 42, 37-48.

Harkouss, R., Mirade, P.S. & Gatellier, P. (2012). Development of a rapid, specific and efficient procedure for the determination of proteolytic activity in dry-cured ham: definition of a new proteolysis index. *Meat Science*, 92, 84–88.

He, F.J. & MacGregor, G.A. (2009). A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *Journal of Human Hypertension*, 23, 363-384.

Leistner, L. (1985). Hurdle technology applied to meat products of the shelf stable product and intermediate moisture food types. *In Simatos D, Multon JL (Eds.), Properties of water in foods in relation to quality and stability (pp. 309-329)*. Dordrecht: Martinus Nijhoff Publishers.

Le Page, J.F., Mirade, P.S. & Daudin, J.D. (2010). Development of a device and method for the time-course estimation of low water fluxes and mean surface water activity of food products during ripening and storage. *Food Research International*, 43, 1180-1186.

Le Page J.F., Daudin J.D. & Mirade P.S. (2012). Time-course estimation of evaporated water fluxes together with mean surface water activity in uncooked pressed cheeses during ripening using a purpose-built micro-bioreactor. *International Dairy Journal*, 22, 66-72.

Lynch, S.M. & Frey, B. (1993). Mechanisms of copper and iron dependent oxidative modification of human low density lipoprotein. *Journal of Lipid Research*, 34, 1745–1751.

Mercier, Y., Gatellier, P., Viau, M., Remignon, H. & Renerre, M. (1998). Effect of dietary fat and vitamin E on lipid and protein oxidation in turkey meat during storage. *Meat Science*, 48, 301–317.

Morzel, M., Gatellier, P., Sayd, T., Renerre, M. & Laville, E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. *Meat Science*, 73(3), 536–543.

Nishida, C., Uauy, R., Kumanyika, S. & Shetty, P. (2004). The Joint WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutrition*, 7, 245-250.

Olivares, A., Navarro, J.L., Salvador, A. & Flores, M. (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. Meat Science, 86, 251-257.

Olivares, A., Navarro, J.L. & Flores, M. (2011). Effect of fat content on aroma generation during processing of dry fermented sausages. *Meat Science*, 87, 264-73.

Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S. & Stadtman, E.R. (1987). Age-related changes in oxidized proteins. *Journal of Biological Chemistry*, 262(12), 5488–5491.

Oqali (2009). Rapport de l'Observatoire et de la qualité de l'Alimentation. Edition 2010. 20 p. (in French)

Rougier, T., Bonazzi, C. & Daudin, J.D. (2007). Modelling incidence of lipid and sodium chloride contents on sorption curves of gelatine in the high humidity range. *Lebensmittel-Wissenschaft-und-Technology*, 40, 1798-1807.

Ruusunen, M. & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science*, 70, 531-541.

Théron, L., Tournayre, P., Kondjoyan, N., Abouelkaram, S., Sante-Lhoutellier, V. & Berdagué, J.L. (2010). Analysis of the volatile profile and identification of odour-active compounds in Bayonne ham. *Meat Science*, 85(3), 453-460.

Tournayre, P. & Berdagué, J.L. (2003-2012). AcquiSniff (version 6.5.5) [Computer software]. *IDDN.FR.001.210006.002.RP.2003.000.30000*. France: Institut National de la Recherche Agronomique.

Weiss, J., Gibis, M., Schuh, V. & Salminen H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, 86, 196-213.