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Theme :

The survival and the sensory effect of the selected strain lactobacillus sakei subsp sakei BK18 during the repining of dry-fermented sausage

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Thanks

God"ﻪﷲthank you for giving us health, willpower and couragewithou work would not have been carried out.First of all we express our gratitude a our

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Halima AchouakFatma

dedicate

With immense honor and great modesty , we dedicate this modest work to :

Our dear parents

Our brothers and sisters

Our families

Our friends and our professors

Halima..Achouak..Fatma

Abstract

Dry-fermented sausages are renowned for their distinct sensory attributes and extended shelf life, which are achieved through the fermentation process involving lactic acid bacteria (LAB) and particularly the strains of *Lactobacillus (Lb.) sakei*. A compared study *in situ* was carried out to understand the survival and the sensory effect of selected strain *Lactobacillus sakei subsp sakei* BK18 on dry-fermented sausages. Started and negative control batches of sausages were prepared under controlled c conditions of temperature and relative humidity for 15 days. The count of LAB and total aerobic mesophilic flora at 30 °C show a correlation during the repining period where the started batch show a high rate comparing to the control. Those results indicate the survival capacity of *Lb. sakei subsp sakei* BK18 and its resistance to hostile conditions of low a_W and osmotic stress. The sensory evaluation, conducted through a questionnaire and public opinion poll, yielded positive results for the Started sausage batch. Most of the participants perceived good sensory attributes, including aspect, smell, color, texture, maturity and acidity. These findings support the potential use of *Lb. sakei subsp sakei* BK18 as a beneficial starter culture to produce fermented and dried sausages, enhancing both safety and sensory quality.

Key words: Sausages , LAB , *Lb. sakei subsp sakei* BK18 , survival, sensory.

Résumé :

Les saucisses fermentées à sec sont réputées pour leurs attributs sensoriels distincts et leur durée de conservation prolongée, qui sont obtenus grâce au processus de fermentation impliquant des bactéries lactiques (LAB) et en particulier les souches de lactobacilles (Lb.) saké. Une étude comparative in situ a été réalisée pour comprendre la survie et l'effet sensoriel de la souche sélectionnée Lactobacillus sakei subsp sakei BK18 sur des saucisses fermentées à sec. Des lots de saucisses commencés et témoins négatifs ont été préparés dans des conditions c contrôlées de température et d'humidité relative pendant 15 jours. Le nombre de LABORATOIRES et la flore mésophile aérobie totale à 30 °C montrent une corrélation pendant la période de repinage où le lot commencé montre un taux élevé par rapport au témoin. Ces résultats indiquent la capacité de survie de Lb. sakei subsp sakei BK18 et sa résistance aux conditions hostiles de faible aW et de stress osmotique. L'évaluation sensorielle, menée au moyen d'un questionnaire et d'un sondage d'opinion publique, a donné des résultats positifs pour le lot de saucisses commencé. La plupart des participants ont perçu

de bons attributs sensoriels, notamment l'aspect, l'odeur, la couleur, la texture, la maturité et l'acidité. Ces résultats appuient l'utilisation potentielle de Lb. sakei subsp sakei BK18 comme culture de démarrage bénéfique pour produire des saucisses fermentées et séchées, améliorant à la fois la sécurité et la qualité sensorielle.

Mots clés : Saucisses , LABORATOIRE , Lb. sous-espèce sakei sakei BK18, survie, sensoriell

الملخص :

تشير النقانق الجافة المخمرة بسماتها الحسية المميزة ومدة صلاحيتها الممتدة ، والتي يتم تحقيقها من خلال عملية التخمير التي تنطوي على بكتيريا حمض اللاكتيك)المخمر(وخاصة سلالت الملبنة)رطل.(ساكي. تم إجراء دراسة مقارنة بي الموقع لدعم البقاء على قيد الحياة والتأثير الحسي للسلالة المختارة الكتوباكيللوس ساكي سوبسب ساكي بي كيه 18 على النقانق الجافة المخمرة. بدأت وأعدت دفعات التحكم الملبية من النقانق تحت ظروف درجة الحرارة والرطوبة النسبية ارتباطا correlation الخاضعة للرقابة لمدة 15 يوما. يظهر عدد المخمر ومجموع النباتات الهوائية المتوسطة بي 30 خلال فترة التكرار حيث تظهر الدفعة االولى معدل مرتفع مقارنة بالتحكم. تشير هذه النتائج إلى قدرة البقاء على قيد الحياة للرطل. ساكي سوبسب ساكي باك 18 ومقاومته للظروف المعادية من انخفاض أو والإلجاء النتاضحي. أسفر التقييم الحسي ، الذي تم إجراؤه من خلال استبيان واستطلاع للرأي العام ، عن نتائج إيجابية لدفعة النقانق التي بدأت. أدرك معظم المشاركين سمات حسية جيدة ، بما بي ذلك الجاذب والرائحة واللون والملمس والنضج والحموضة. تدعم هذه النتائج االستخدام المحتمل للرطل. ساكي سوبسب ساكي بي كيه 18 كثقافة بادئة مفيدة إلنتاج النقانق المخمرة والمجففة ، مما يعزز السلامة والجودة الحسية.

الكلمات المفتاحية: النقانق ، المخمر ، رطل. ساكي سوبسب ساكي بي كيه 18, فجاة, حسي.

List of Figures

Figure.1. General flow chart for starter culture fermentated sausage production

Figure.2. Most important factors affecting the viability of probiotics (starter culture) in fermented sausage

Figure.3. Pictures. Colony morphology (a), Gram staining cell morphology (b) and scanning electron micrograph (c) of strain CMC 1-8.

Figure.4. Phylogenetic tree drawn from the rpoB gene sequences of the main species belonging to the Lb. sakei clade.

Figure.5. Diagram of dry-fermented sausages preparation.

Figure.6. The steps of preparation sausages

Figure.7. Macroscopic and microscopic aspect of Lb. sakei subsp sakei BK18

Figure.8. Evolution of LAB during the ripening sausages

Figure.9.Evolution of total aerobic mesophilic flora during the sausages

Figure.10.Comparative Sensory-evaluation between starter-inoculated and control sausages.

List of abbreviations

MRS: Man-Rogosa-Sharpe

PCA: Plant Count Ager

Na Cl: sodium chloride

LAB: Lactic Acid Bacteria

TAMF:total aerobic mesophilic flora

SM:Standard mean

SE:Standard error

measuring units:

C°: degree Celsius

mL : milliliter

The strain:

Lb.sakei :Lactobacillus sakei

L. monocytogenes : Listeria monocytogenes

RA:rheumatoid arthritis

CIA:collagen induced arthritis

Sommer

Chapter I. Generalities about sausages

1. General information about sausage making… 3

2. Geographical production 4

3. The main ingredients in sausage 6

3.1. Sodium Chloride 6

3.2. Fat… 7

3.3. Lactic acid bacteria starter without Probiotics 4 7

3.4. Classification 9

5 .The main types of sausages 9

5.1. Fresh Sausages 9

5.1.1. Fresh Pork Sausage 9

5.1.2. Fresh Beef Sausage 10

5.2. Dry and Semidry Sausages 10

6. Fermentation in sausage 10

7. Microorganisms in sausage and their pathogenicity… 12

8. Lactic acid bacteria and Lactobacillus sakei and their benefits in sausages 13

Chapter II. Lactobacillus (Lb.) sakei

II.1. General information about Lb. sakei 14

II.1.1. Presentation 14

II.1.2. Characteristics 14

II.2. Morphology…..... 15

II.3. Culture of Lactobacillus sakei..... 15

II.3.1. Nutrition sources: II.3.1.1.3. Use of Carbon Sources..... 15

II.3.1.1.4. Other Nutrients 17

III.3.2 Ability to resist to various stresses 17

II.4Intra-species diversity of Lb. sakei 18

III.3.1The Lb. sakei Clade.....18

II.4The Genomic Diversity among Lb. sakei Strains..... 19

II.4The features of Lactobacillus sakei.....20

II.3.1Nutritional effect… 20

II.3.1Therapeutic effect…….,…..... 21

II.3.1 Biotechnology features for dry fermented meat products..... 22

II.3.1.1. Safty of starter culture..... 22

II.3.2.2.Maintining the color..... 22

II.3.1.1. Enzyme activities..... 23

II.3.1.2. Lb.sakei as a starter and functional starter..... 23

II.3.2. The use of Lb. sakei as a Starter for fermented sausage 25

Material and Methods

I.1. The objective of the study 28

I.2. Starter culture..... 28

I.3. Preparation of the culture for inoculation..... 28

I.4. Manufacturing of the Sausages 29

I.5. Microbiological Analyses 30

I.6. Sensory analysis 31

I.7. Statistical analysis.....32

I.8. Financial support… 32

III. Results and Discussion

III.1. Macroscopic and microscopic aspect of Lb. sakei subsp sakei BK18.... 34

III.2. Evaluation of the microbiological changes during the repining…..... 35

III.2.1. Enumeration of total aerobic mesophilic flora (AMF) and LAB… 35

III.3. Sensory evaluation.....38

Conclusion..... 43

Introduction:

Dry fermented sausages are popular meat products known for their unique sensory characteristics and extended shelf life. The fermentation process involved in their production relies on the activity of lactic acid bacteria (LAB), particularly strains of *Lactobacillus (Lb.) sakei* strains especially for Mediterranean countries. LAB and *Lb. sakei* species play a crucial role in enhancing flavor, texture, and safety through the production of lactic acid and various metabolites.

The survival and sensory properties of *Lb. sakei* strains during the ripening of dry fermented sausages has a great interest for researchers and food industry professionals. Understanding the behavior and performance of these strains under different processing conditions can contribute to the development of high-quality products with improved sensory attributes.

The survival of *Lb. sakei* strains during fermentation is influenced by several factors, including pH, temperature, salt content, and the competition with other microorganisms present in the sausage. The ability of these strains to withstand these environmental challenges is crucial for their dominance and successful fermentation.

Furthermore, the sensory attributes of dry fermented sausages, such as aroma, taste, and texture, are directly influenced by the metabolic activity of LAB. Selected starters of *Lb. sakei* strains for sausage fermentation can have a significant impact on the overall sensory profile of the final product. Mixed starters of LAB and coagulase negative cocci (CNC) may contribute on flavors and textures, leading to the development of sausages with distinct regional or artisanal characteristics.

The aime of this study is to investigate the survival and sensory effect selected strain of *Lb. sakei subsp. sakei* BK18 during the ripening process of dry fermented sausages. By evaluating the survival rates of this particular strain under varying conditions and examining its impact on the sensory attributes of the sausages, valuable insights can be gained regarding the potential for optimizing fermentation processes and enhancing product quality.

Chapter I. Generalities about sausages

1. General information about sausage making :

Traditional fermented meat production has a long production history and is very popular in different parts of the world. Variations in production conditions often contribute to specificity in texture and flavour **(Sarkadi et *al*., 2017).** Due to the high demand and popularity of fermented meat products, small traditional production was often replaced by large industrial production. However, obtaining a final product that meets the texture and flavour of the traditional product is still a challenge for the meat industry **(Franciosa *al*., 2018).**

Attributes of fermented meat products are derived from extensive protein changes occurring in fermentation and maturation processes. Proteolysis is one of the most important biochemical changes that influences both texture and flavour development due to the formation of several low-molecular-mass compounds, including peptides, free amino acids, aldehydes, organic acids and amines. **(Hughes et *al*., 2002).** Generally, it is known that hydrolysis of meat proteins generates polypeptides by endogenous muscle enzymes, such as cathepsins and calpains and proteasome. **(Zhou et *al*., 2021).** The polypeptides can be further degraded to smaller peptides and free amino acids by the action of peptidases and aminopeptidases from both muscle and microorganisms. **(López et *al* ., 2015.).** Free amino acids released during ripening directly contribute to the basic taste of dry fermented sausages. Amino acids are precursors of many volatile compounds thus indirectly contributing to the development of the typical aroma of the final meat product**. (Zhou et *al*., 2021).** It is well known that technological conditions during fermentation may determine and/or regulate protein degradation. **(Juraj et *al*., 2017).** Furthermore, in order to shorten the ripening time, ensure color development, enhance flavor, improve product safety, and preserve the original sensory quality of the products, starter cultures are frequently used in industrial production .**(Sarkadi et *al*., 2017).**

The production of the first sausages is thought to have taken place in ancient Greece, as evidenced by quotes from Homer who described sausages as a delicacy of minced meat and spices. Today, sausages belong to the largest group of meat products available in a wide range of types and with different commercial names. Numerous requests from consumers initiate the production of new types of sausages, with different composition and additives to improve their nutritional and health safety, in accordance with modern technological production process. **(Hammes et *al*., 1998).**

2.Geographical production:

- In Latin America, a variety of typical chorizos or chorizo-like sausages introduced by European immigrant colonizers, usually resembling Italian and Spanish furthermore Portuguese-style fresh/semidried chorizos, are also prepared and consumed.**(González et *al*., 2014) .** These can be considered creole meat products that have evolved over time into traditional American varieties **(Mateo et *al*., 2009) .**

At present, they constitute an important part of local economies and traditions in Latin America. However, their production has increasingly become more industrialized **(González et *al*., 2014)** Moreover, due to the historical Spanish influence, varieties of chorizo and langgonisang (chorizo -like sausage) are found in the Philippines **(Briones et *al*., 2013)**, and Portuguese influenced varieties of chouriços are found in African (Mozambique, Cape Verde) and Asian (Goa, Timor) regions. Due to more recent migration, chorizos/chouriços can be easily found and are popular in countries around the world, classified as ethnic food, such as in the USA or South Africa. Chorizo sausages in Latin America are normally fresh/semidried and their definition seems to be more flexible than in Spain or Portugal. Mexico is probably the country with the chorizo most similar to the Spanish fresh semidried chorizo. In Argentina, Bolivia, Colombia and Uruguay, Chorizo is used as a generic name for a wide range of fresh/semidry sausages made from coarsely minced meat **(Allen., 2015).** On the other hand, some Latin American chorizo-like sausages are called salchicha or Longaniza, for example the Chilean Longaniza de Chillán**. (Camerati et *al*., 2017).** In Peru, the names chorizo and salchicha appear to be used for regional fresh/semidried chorizo like sausages, depending more on the regional practices than on technology. In Brazil, what is usually referred to as chorizo in other Latin American countries is called linguiça, while Brazilian chouriço refers to a blood sausage. **(Allen., 2015).**

- In Italy, pork sausages, labelled on the market as ‘salsiccia’, are a very popular food. The term ‘salsiccia’ refers to very different products, from fresh unripened sausages to dry fermented sausages **(Consigliere et *al*., 2018).** Fresh salsiccia is a little sausage (about 100 g), made by a ground mixture of pork lean meat and fat, with salt and variable spice mixes; it is stuffed in natural casings and commonly sold in strings, at refrigeration temperature. Fresh unripened sausages have an intrinsic perishable nature, due to several factors, such as high water and nutrient content of the ground matrix and absence of a

lactic acid protective microflora. As a result, the shelf life of fresh sausages is usually short (7–10 days). The habit of consuming fresh sausages without cooking or not thoroughly cooked, not uncommon in Italy as well as in other countries **(Rossi et *al*., 2011),** is a matter of concern for public health. In particular, the prevalence of *L. monocytogenes .(L)* in these products is not negligible **(De Cesare et *al*., 2007).** This can be related with their high pH and a_W values and with the widespread diffusion of the pathogen in pork meat processing facilities **(Meloni ., 2015).** Listeriosis is an important food disease worldwide **(Desai et *al*., 2019).** A statistically significant increasing trend of confirmed listeriosis cases in the EU has been observed in 2009–2018, and consumption of raw or undercooked fresh salsiccia can represent a vehicle for human listeriosis **(Franciosa et *al*., 2020).**

In Europe, dry fermented sausages have a long tradition originating from Mediterranean countries during Roman times. Processing conditions, as well as ingredients and additives, vary among the different types of fermented sausages **(Gardini et *al*., 2001)**. In fact, 'typical' foods of any region or area have their own peculiar characteristics that are deeply rooted in tradition and linked to the territory and which arise from the use of local ingredients and specific production techniques **(Aquilanti et *al*., 2007).** The production process begins with small pieces of meat and fat that are minced; salt and spices and in some cases sugar, herbs and/or other ingredients are then added. The homogenized mixture is then stuffed into casings, and undergoes fermentation and drying. European legislation, under Reg. EC 1333/2008 (and subsequent modifications), allows the use of nitrate and nitrite as preservatives, unless subject to other regulations for protected denomination of origin (PDO) products **(Aquilanti et *al*., 2016).** The qualitative characteristics of fermented sausages are known to be largely dependent on the quality of the ingredients and raw materials, the specific conditions of the processing and ripening, and the composition of the microbial population **(Aquilanti et *al*., 2007).** Pathogenic and spoilage bacteria are inhibited; consequently, the final product has an increased shelf-life **(Hugas et *al* ., 1997).**

Meat fermentations are complex microbial ecosystems in which bacteria, yeasts and molds coexist. Considerable microbial diversity is observed during the fermentation process and is evidenced by the presence of several species belonging to different genera, but also strains of the same species. Through fermentation, highly perishable raw materials, such as meat and fat, are transformed in microbiologically stable final products, characterized by a defined sensory profile, enhanced due to sodium chloride supplementation and to the

drying process **(Cocolin et *al*., 2011).** Changes that occur during fermentation and drying influence the aroma development in fermented sausages **(Flores et *al*., 2004).**

Many typical fermented meat products are still produced with traditional technologies without selected starters. However, in the modern sausage production, the use of starter cultures is becoming more frequent to guarantee safety and to standardize product properties, for example consistent flavor and color and shorter ripening time. **(Cocolin et *al*., 2001).**

3.The main ingredients in sausage:

3.1. Sodium Chloride:

Sodium chloride is an essential multi-functional ingredient in dry fermented sausages. Sodium chloride is involved in myofibrillar protein solubilization, enhancing water binding and water retention capacity (thus resulting in the formation of a desirable gel texture), decreasing water activity (aw; thus, controlling the growth of pathogen microorganisms), promoting characteristic sensory attributes, and finally, controlling biochemical and enzymatic reactions during ripening **(Pires et *al*., 2017).** However, the global daily sodium intake, estimated at 9–12 g, is double the recommended maximum levels **(Pateiro et *al* ., 2021).** A diet high in sodium increases the risk of developing cardiovascular diseases (especially hypertension) and it is also considered to be a risk factor for other diseases (such as obesity, kidney stones, and some cancers) **(Appel et *al*., 2010)**, particularly for meat products, in which the content can achieve 5 g/100 g **(Vidal et *al*., 2021).** Therefore, reducing sodium content in fermented meat products may be useful to reduce the daily sodium intake for meat products consumers. However, it is important to note that it represents a major challenge for the meat industry, since sodium reduction can cause a loss of quality and lead to safety concerns. A low sodium content could also affect flavor and taste due to protein oxidation, lipid oxidation, and lipolysis, which are the most remarkable characteristics and important indexes for quality evaluation**.** Sodium reduction in dry fermented sausages formulation could increase the activity of proteolytic enzymes, resulting in a softer and more brittle texture due to the higher degradation of myofibrillar proteins evaluation **(Toldrá et *al*., 2006).**. Furthermore, the inhibition of pathogenic microorganisms could not be guaranteed. Nowadays, in order to reduce health risks, many authors have studied the effects of sodium reduction as well as different sodium substitutes in technological properties and bacterial counts in fermented sausages **(Noelí et *al*., 2022).**

3.2. Fat:

Fat is a main component in the production of meat products due to its importance in the sensory and microbial quality. However, meat products are important dietary sources of saturated fatty acids (SFAs) and cholesterol that have been indicated as a risk factor for the development of severe illnesses **(Kaur et *al*., 2019).** Consequently, the reformulation became necessary to produce healthier and functional meat products. The professionals of the meat industry and researchers have put emphasis on the study, production, and commercialization of healthier products, where the reduction of saturated fat is achieved **(Barone et *al*., 2021)** . Developing solutions to produce healthier products with the preservation of flavor and consumer acceptability and expectations represents an important challenge **(Shan et *al*., 2017).**

3.3. Lactic acid bacteria starter without Probiotics:

Dry fermented sausages have a unique texture and typical fermentation-derived flavors. The variability in the autochthonous microbiota is one of the key aspects that dictate the characteristics and sensory attributes of traditionally fermented sausages. However, these traditionally fermented sausages generally undergo spontaneous fermentation; therefore, the quality and safety of the meat products may be affected among batches **(Dos Santos et *al*., 2019).** In this scenario, standardizing the production process with selected microorganisms (used as starter culture) in the traditional dried fermented sausage production is a fundamental strategy. Microorganisms isolated from traditional meat products are promising candidates for meat starter culture (usually inoculated at a rate of 10^6 UFC/g of meat batter) since they have good adaptability to the ecological niche of fermented meats **(Vermassen et *al*., 2014).** It is important to mention that safety assessment is necessary to support the selection of isolated candidates for probiotic sausages production. Applicable tests aim to characterize the presence of virulence factors, production of biogenic amines, hemolytic activities, and susceptibility to antibiotics. Nowadays, starter cultures play a role beyond quality in dry fermented sausages by providing additional health benefits. This condition is particularly relevant in the case of probiotic microorganisms. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" **(Hill et *al*., 2014).** Several beneficial effects have been demonstrated from the regular consumption of probiotics, such as immune system stimulation, intestinal mucosa barrier, reduction in diarrhea occurrence, stimulation of intestinal motility, relief of lactose intolerance

symptoms (due to better use of this disaccharide), production of digestive and protective enzymes, reduction of constipation, colon cancer prevention or suppression, and anti-carcinogenic effects in vivo **(Ambalam et** *al* **., 2016).**

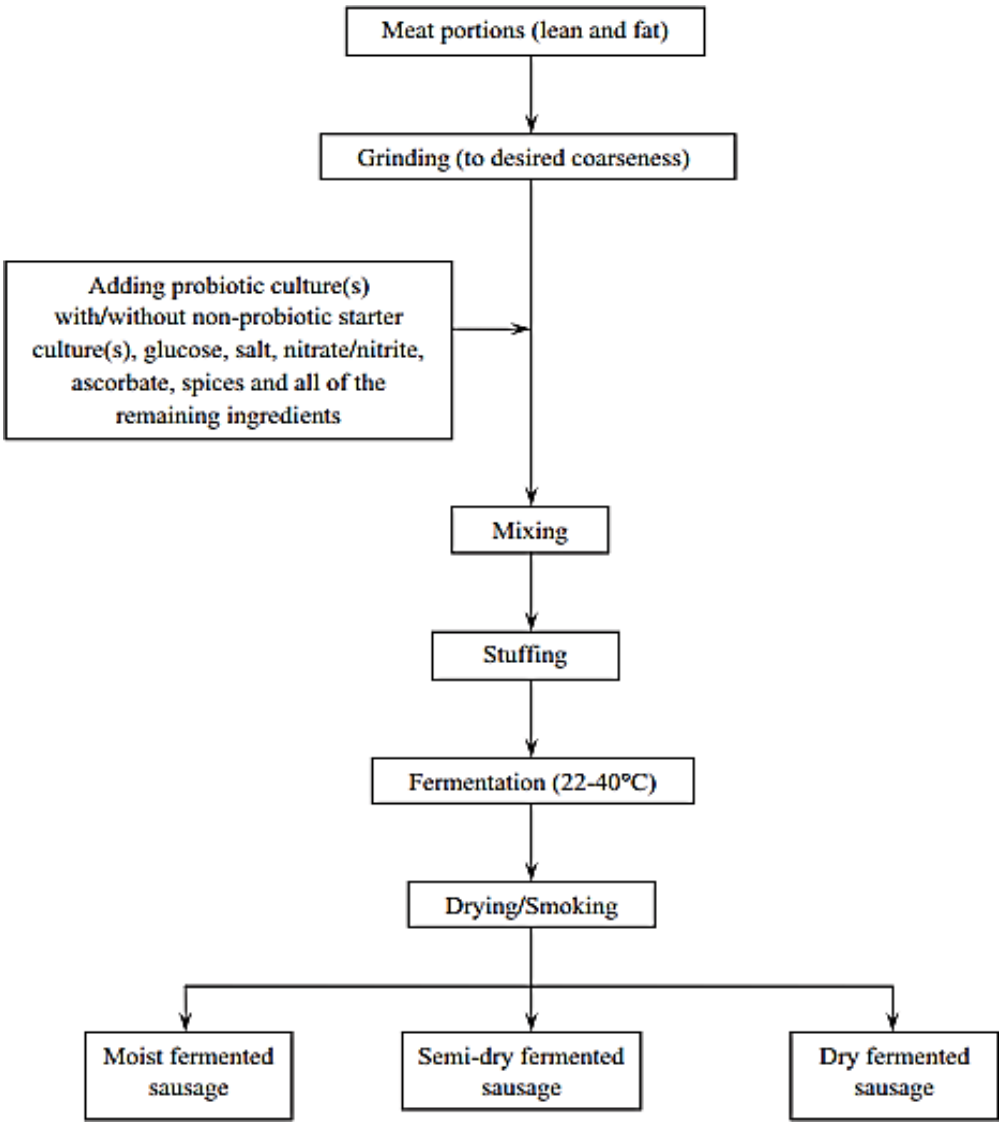
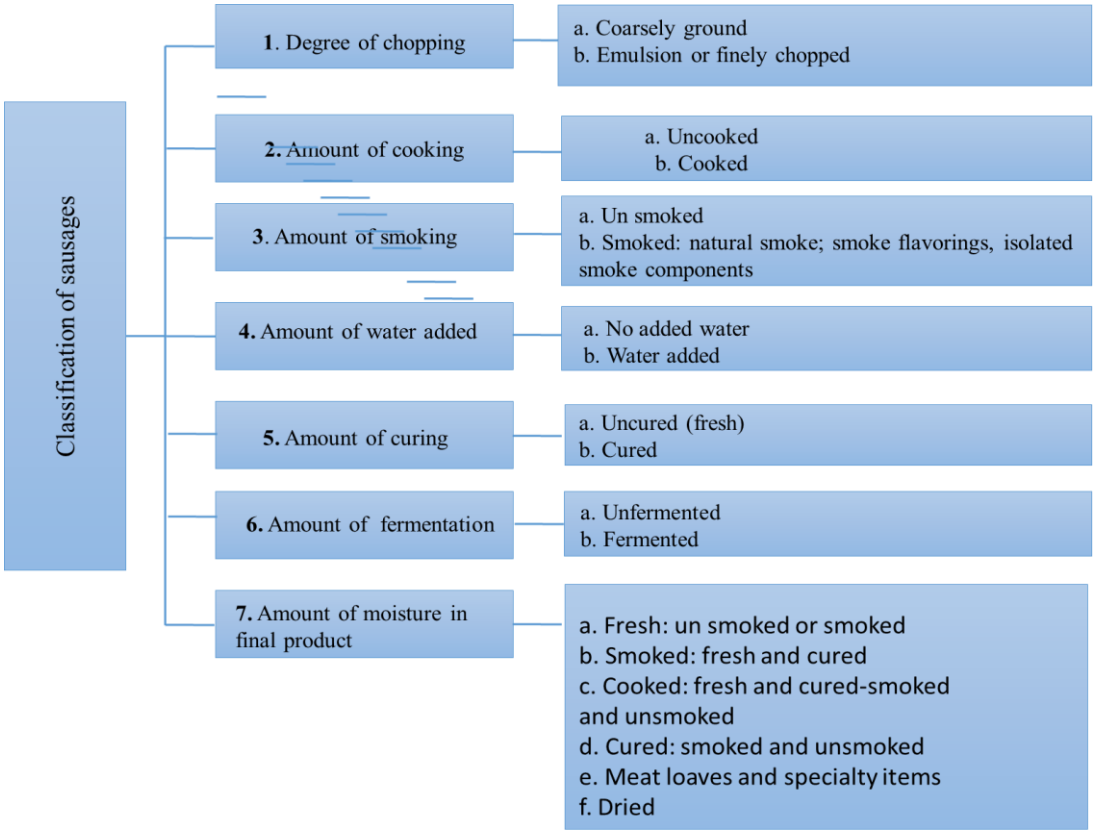


Fig.1. General flow chart for starter culture fermentated sausage production

4.Classification:



(Pearson et *al*., 1996)

5.The main types of sausages:

5.1. Fresh Sausages:

Made of fresh, uncured meat, generally cuts of fresh pork and sometimes beef, their taste, texture, tenderness, and color are directly related to the ratio of fat to lean. Trimmings from primal cuts such as pork loins, hams, and shoulders are often used. They must be kept under refrigeration and thoroughly cooked before serving. Bratwurst is in this class. If a mixture of pork and other meats in hog casings or smaller casings are used, they need not be treated for trichinae. Mixtures in casings larger than hog casings must be treated for trichinae. **(Pearson et *al*., 1996).**

5.1.1. Fresh Pork Sausage:

This is made from fresh or frozen pork or both including mechanically deboned pork, but not including pork by-products. The finished product cannot contain over 50% of total fat. Water and/or ice can be added up to 3% of the total to facilitate chopping and mixing. **(Pearson et *al*., 1996).**

5.1.2. Fresh Beef Sausage:

The same as fresh pork sausage except beef is substituted for pork and the finished product cannot contain over 30% fat. **(Pearson et *al*.,1996).**

5.2. Dry and Semidry Sausages

These products are produced by fermentation due to either "backslapping"(natural) or addition of a lactic acid producing culture. The production of lactic acid not only aids in preservation by lowering the pH and inhibiting the growth of undesirable microorganisms but adds a tangy flavor. Meter mixing the meat ingredients with the spices, cure, and culture, the meat is held in a curing cooler until the desired acidity is achieved. Then the meat is stuffed into casings and air dried under carefully controlled drying conditions. Some products are lightly smoked during preliminary drying, but the essential step is air drying. Meat is stuffed into casings and air dried under carefully controlled drying conditions. Some products are lightly smoked during preliminary drying, but the essential step is air drying. **(Pearson et *al*.,1996).**

6. Fermentation in sausage:

Fermented sausage is one of the common fermented meat products. Due to its unique flavor and taste, it is favored by worldwide consumers **(Liu et *al*., 2021)** It is usually stored and transported at normal temperature and can be eaten directly without cooking. However, many safety risks occur during the processing and storage of fermented sausages, such as oxidative rancidity, pathogenic bacteria and an excessive content of biological amines **(Luan et *al*., 2021)** Proteins in meat could be converted into biogenic amines under the action of amino acid decarboxylase secreted by microorganisms in the fermentation process **(Algahtani et *al*., 2020).** Meanwhile, biogenic amines are not volatile and have high thermal stability. Thus, the biogenic amine content is a common phenomenon in fermented sausages. A certain amount of biogenic amines is indispensable for human beings, but they are potentially toxic to the human body and even cause obvious adverse reactions when an individual ingests excessively. Therefore, the biogenic amine content is regarded as an important indicator for the safety assessment of fermented meat products, and it has been attracting attention from an increasing number of related research groups **(Jiaa et *al*., 2020).** Casings play an important role in the industrial production of fermented sausage **(Chen et *al*., 2019).** There are a variety of casings with different functional characteristics in market at present according to the different demands for meat

packaging material in different countries and regions. Casings can be further divided into natural casings and collagen casings in terms of edibility. Natural casings are made from the fresh intestines of healthy animals after some processing, such as pig and sheep **(Patricia et *al*., 2020).** But it cannot be adapted to large-scale industrialized mass production due to their single source and poor processing performance. Collagen casing is an edible artificial casing, which is made from animal dermis. It has stable performance during industrial filling, which can produce products of uniform specifications, reduce the generation of defective products and achieve maximum benefits **(Serio et** al **., 2020).** The maturation process of fermented sausage is easily affected by the type of sausage casing. At present, most studies focus on the comparison of structural properties between collagen casings and natural casings **(Hilbig et *al*., 2020).** However, there is little information about the effects of collagen casing on fermented sausage quality characteristics **(Luan et *al*., 2021)**. Serio *et al*. showed that different types of casings had a great influence on fermented sausages quality, which may affect biogenic amine content and the production of some volatile flavor compounds through affecting proteolysis in ripening time **(Serio et** al **., 2020).**

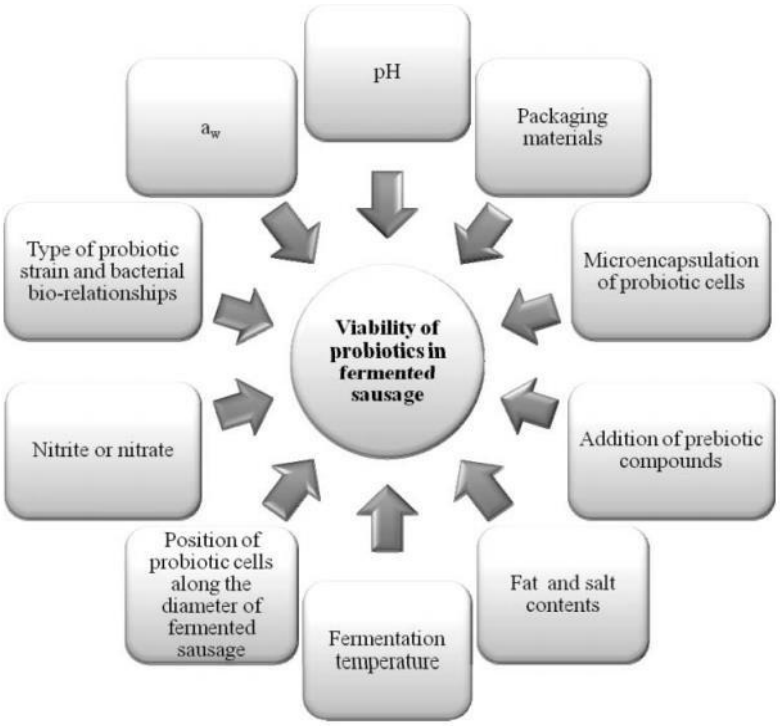


Fig.2. Most important factors affecting the viability of probiotics (starter culture) in fermented sausage

7. Microorganisms in sausage and their pathogenicity:

Fermentation is one of the oldest preservation methods known to human society and in particular to people living in Mediterranean countries **(Oliveira., et *al*., 2018).** Fermentative processes have primarily been used for the conservation of food, using bacteria that convert sugars present in the raw product into products such as lactic acid. The resulting reduction in pH leads to impaired development of a range of microorganisms and thus contributes to the assurance of a microbiologically healthy product. Fermentation of sausage utilizing indigenous microflora still exists. **(Rantsiou et *al*., 2005)**. For example, in Greece, Italy, and Spain, efforts are still dedicated to the study of traditional production methods.**(Comi et *al*., 2004)**. In such products, LAB and coagulase-negative cocci (CNC) are the most active of the indigenous microorganisms involved. **(Lucke., 2000)**. However, with the increasing interest in biological preservation techniques, the application of LAB as starter and/or protective cultures is attracting growing interest.**(Devleighere et *al*., 2004).** In particular, the use of starter cultures for sausage production is becoming increasingly necessary to improve safety, to standardize product propert inies including consistent flavor and color, and to shorten ripening times. **(Laranjo et *al*., 2019).**

The commonly favored microorganisms used as starters tend to be species of *Lactobacillus, Pediococcus, Micrococcus,* and *Staphylococcus*. **(Hammes., et *al*., 1994)** However, fungal starters have also been used to ferment such meat products. **(Toledo et *al*., 1997).**

In contrast, some processors have endeavored to accelerate the ripening process by using proteases and lipases from a range of sources. **(Bruna et *al*., 2000a, 2000b).** Several studies have examined the effect of using combinations of starter cultures for the ripening of fermented sausage. **(Candogan et *al*., 2008).** In particular, staphylococci and micrococci have been used in combination with LAB due to their ability to contribute to flavor, mainly through lipolysis activities, and also due to their abilities to reduce nitrate and produce catalase which could contribute also to fix and obtain the typical color of the final product. **(Nychas., et *al* ., 1990)** The manufacture of dry-fermented sausage is considered to occur in three phases: formulation (mixing of ingredients), fermentation, and ripening/drying. **(Fernandez et *al*., 2000)** During the ripening of dry-fermented sausage, microbiological, biochemical, and sensorial changes take place that are closely related to the activity of the dominant microorganisms present in the sausage matrix. The LAB most

frequently isolated from dry sausage are *Lactobacillus sakei*, *Lactobacillus curvatus,* and *Lactobacillus plantarum*. **(Hammes et *al*., 1990).** *Lactobacillus sakei* is the b acterials pieces most frequently p referred in Western Europe as a meat starter culture, due to its ability to compete against other bacteria during sausage fermentation. **(Zagorec et *al*., 2017).**

Furthermore, Hufner and Hertel (2008) demonstrated that (Lb) retained its high performance in sausage fermentation even after sub lethal stress treatments were applied prior to inoculation into the meat matrix. Moreover, Leroy and De Vuyst (1999, 2005) have demonstrated that the bacteriocin-producing strain (Lb) CTC494 may represent both a promising protective and functional sausage starter culture. **(Mejri et *al*., 2017).**

8. Lactic acid bacteria and lactobacillus sakei and their benefits in sausages:

In the evaluation of the growth/inactivation of (L) throughout the processing of "salchichón", the effect of lactic acid bacteria (LAB) should be considered because selected LAB strains have demonstrated antimicrobial effect against this pathogenic bacterium **(Pedonese et *al*., 2020).** Among LAB, Lactobacillus sakei has a technological use in the preservation of several dry-cured fermented sausages due to its capacity to produce organic acids, hydrogen peroxide, and bacteriocins **(Chaillou et *al*., 2005).** In fact, L. sakei has shown protective antimicrobial effect against both pathogenic and spoiler microorganisms in meat products (**Gelinski et *al*., 2019).**In addition to the evaluation growth/inactivation of (L), the effect of processing of "salchichón" on the evolution of a natural microbial population should be determined, because unusual development of some microbial groups may lead to irregular microbial quality of the sausages and even spoilage during ripening. For example, growth of some Enterobacteriaceae strains throughout ripening in dry-cured meat products has been reported **(García et *al* ., 2000),** which may reduce the microbiological quality of the sausage pieces. With the aim of evaluating potential food safety implications, "salchichón" spiked with selected strains of (Lb) was challenge-tested with low and high levels of (L). In addition, the effect of dry-cured fermented sausage "salchichón" processing on the evolution of the natural microbial population of this product was evaluated **(Martín et *al*., 2021).**

Chapter II. *Lactobacillus (Lb.) sakei*

II.1. General information about *Lb. sakei*:

II.1.1. Presentation:

Lb. sakei is a non-pathogenic lactic acid bacterium, which was first described as a contaminant of Japanese rice wine "sake" **(Katagiri et *al*.,1934)**. It was also found in various fermented products, such as sourdoughs and fermented cabbage. *Lactobacillus sakei* is characteristic for meat products. It was found naturally on fresh meat and fish and fermented sausage. They are used as starters for fermentation of fish and meat product **(Bjerke et *al*., 2019).**

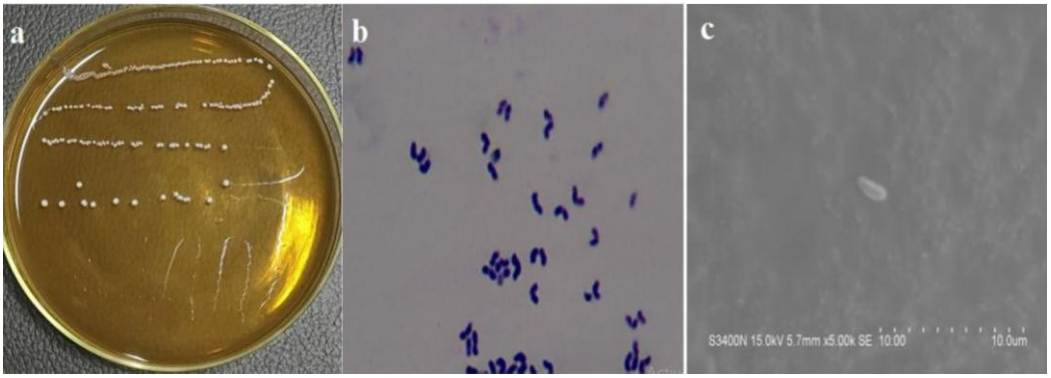
II.1.2. Characteristics :

Lactobacillus sakei is able to colonize many different habitats. It has often been isolated from food of vegetable sources like various flours, sourdoughs, or fermented cabbage **(Coda et *al*., 2017)**, but is systematically associated with meat products and often with seafood **(Najjari et *al* ., 2016)**. Because of its presence in many foodstuffs, we can therefore consider this lactic acid bacterium (LAB) as belonging to the human diet. Consequently, it has also been isolated from human feces **(Chiaramonte et *al*., 2009)**. Nevertheless, the gastrointestinal tract does not appear as its preferred environment **(Chiaramonte et *al*., 2010)**. Indeed, *Lb. sakei* is the emblematic LAB of meat products in particular of raw meat products stored at low temperature and under vacuum packaging and of fermented sausages. This results from its metabolic activities and phenotypic traits that are particularly well adapted to its growth and survival under the conditions encountered during meat storage and processing **(Chaillou et *al*.,2005)**.

Since its discovery, many studies have aimed at understanding the physiology of this LAB, focusing on its remarkable adaptation to the meat environment and to optimize its use for biopreservation or as starter for the fermentation of sausages. Several metabolic traits have been described in detail, including its ability to use various nutrients present in meat that confer it a selective advantage. *Lb. sakei* is a psychotropic and facultative anaerobic species, it is favored by oxygen-depleted environments or vacuum packaging and cold temperature, conditions prevalent in meat product storage. Consequently, it has also often been identified in large amounts in spoiled products **(Doulgeraki et *al*.,2012).**

II.2. Morphology :

Morphological observation of single colony isolated and purified from stinky mandarin fish was shown in (picture 1a) . Once ending culture process, it was showed that the colony was round with neat edges, milky white or milky yellow, raised surface, moist and smooth, diameter of 1.8-2.0 mm, accompanied by a sour taste. The Gram staining and scanning electron microscope observations of the strain were shown in (Figure 1b) and (Figure 1c), respectively. Gram staining was positive, and the cell morphology was short rod-shaped, paired or short-chain without spores **(Yong-Xiang et *al*.,2023).**



Pictures . Colony morphology (a), Gram staining cell morphology (b) and scanning electron micrograph (c) of strain CMC 1-8.

II.3. Culture of *Lactobacillus sakei*:

For the normal growth of LAB, rich bacterial media containing various compounds such as amino acids, vitamins, minerals, and other compounds is required. The standard recommended media for cultivation of lactic acid bacteria is MRS (De Man, Rogosa and Sharpe), which contains meat extract, yeast extract, peptone, and growth components. However, the growth of LAB in MRS medium can be inhibited due to production of organic acids and nutrients limitation **(Leroy et *al*., 2001)**. In the present times, there is a growing demand for production of food ingredients, including bacterial starter cultures. There is a great scientific interest in development of culture media for bacterial concentrates **(Aspmo et *al*., 2005).**

II.3.1. Nutrition sources:

The main energy sources are sugars: hexose fermentation is *homolactic* while pentoses (such as ribose) are fermented through the heterolactic pathway **(Rimaux et *al*.,2011, check out if there is: Rimaux et *al*.,2011)**. Nevertheless, the ability of the species to dominate the microbiota of fermented sausages for several weeks, when the hexoses are depleted after few

days from the production, underlines its ability to use others substrates to obtain energy for growth and survival **(Cocconcelli et** *al* **.,2010).**

II.3.1.1. Ability to use nutrients encountered in meat to produce metabolites:

II.3.1.1.1. Use of Amino-Acids:

Meat is a rich substrate providing amino acids and peptides issued from proteolysis of myofibrillar and sarcoplasmic proteins. In *Lb. sakei*, amino acid prototrophy is strain-dependent **(Lauret et** *al***.,1996)**. A transcriptomic study showed that in the presence of meat proteins *Lb. sakei* up-regulated genes encoding oligopeptide transporters and intracellular peptidases **(Xu H.Q et** *al***.,2014)**. indicating the ability of this species to take advantage of meat nutrients.

II.3.1.1.2. Use of arginine:

An abundant amino acid present in meat **(Aristoy et** *al* **., 1998)**, is well documented in *Lb. sakei*, it involves the arginine deiminase pathway (ADI) that produces ornithine, ammonia, and carbon dioxide and concomitantly generates ATP. In *Lb. sakei*, the ADI pathway is encoded by the *arcABCTDR*, *PTP* gene cluster **(Rimaux et** *al***., 2013)**. Arginine utilization by *Lb. sakei* is submitted to catabolite repression **(Zuñiga et** *al***.,1998)**, is sensitive to pH **(Rimaux et** *al***.,2012)**, and is induced by the presence of arginine and by anaerobiosis **(Champomier-Vergès et** *al***., 1999)**, correlating to conditions encountered during meat storage. Although the use of arginine does not confer an advantage for growth **(Zúñiga et** *al***., 2002)** , it enhances the survival of *Lb. sakei* because of the ATP it generates and not because of ammonia production **(Zúñiga et** *al***., 2002)**. Thus, this function participates to *Lb. sakei* adaptation to meat.

II.3.1.1.3. Use of Carbon Sources:

Conversely to its richness in amino acids and peptides, meat is a relatively poor substrate for sugars. In laboratory conditions, *Lb. sakei* strains preferably ferment glucose, *N*-acetyl-D-glucosamine, sucrose, fructose, and mannose, which are transported by the PEP dependent phosphotransferase system (PTS) **(Chaillou et** *al***., 2005)**. However, these carbon sources are not very abundant in meat. Ribose is one of the carbohydrates present in raw meat, and *Lb. sakei* has been shown to use it through an ATP-dependent system **(Lauret et** *al***., 1996)**. Several uncommon aspects of ribose utilization by *Lb. sakei* were reported. The gene repertoire of *Lb. sakei* also suggested the ability of this species to use alternative carbon sources such as nucleosides or *N*-acetyl-neuraminic acid that are present in meat **(Chaillou et** *al***., 2005**).

The utilization of the pentose moiety of adenosine and inosine as a carbon source was confirmed experimentally, proving that *Lb. sakei* can indeed take advantage of alternative molecules for its carbon metabolomic study catabolism in meat **(Rimaux et *al*., 2011)**. A performed in laboratory medium showed that nutrient utilization varies depending on the growth phase: first, *Lb. sakei* consumed glucose, fructose, carnitine, tryptophan, and malic acid from the medium, and then it used trehalose, citric acid, and lysine that had accumulated into the cells **(Lee et *al*., 2017)**.

II.3.1.1.4. Other Nutrients:

Meat, particularly red meat, is known to be a source of iron, being part of the heme prosthetic group of hemoglobin and myoglobin. The analysis of the genome sequence of *Lb. sakei* suggested the presence of several transporters putatively involved in iron or heme uptake **(Chaillou et *al*., 2005**). As well, *Lb. sakei* is one of the rare LAB that possess a heme-dependent catalase, although it is unable to synthesize heme **(Hertelet et *al* ., 1998)**. Therefore, the ability to transport iron/heme and iron/heme-containing molecules was investigated. The catalase was active when *Lb. sakei* cells were cultivated in a medium supplemented with hematin, myoglobin, or hemoglobin, showing that this species could indeed transport heme or heme-carrier molecules **(Duhutrel et *al* .,2010)**. In addition, heminic compounds supplementation of the growth medium enhanced the survival of *Lb. sakei* during stationary phase. A microscopy approach, based on electron energy loss spectroscopy analysis and nano-scale secondary-ion mass spectrometry, clearly showed that heminic compounds were internalized by *Lb. sakei*. However, iron ($FeCl_3$) did not enter the cells **(Duhutrel et *al* .,2010)**.

II.3.2. Ability to resist to various stresses:

The analysis of *Lb. sakei* 23K whole genome sequence revealed the large genetic equipment for resisting harsh conditions encountered during meat storage and meat processing **(Chaillou et *al*., 2005)**. One of the stresses occurring during meat processing is the oxidative stress that can occur during meat mixing or during storage under oxygen enriched modified atmospheres. The redundancy of genes potentially involved in oxidative stress response (thioredoxins and thioredoxin reductases) and the presence of a putative glutathione/glutaredoxine/glutathione reductase system may contribute to *Lb. sakei* resistance to oxidative stress. In addition, as mentioned above, *Lb. sakei* strains possess a heme-dependent catalase, encoded by *katA*, that can be active when heme is present in the medium and transported into the cell. A mangenese/iron superoxide dismutase

gene *sodA* is also present in the genome *sodA*, in association with the catalase, enables the detoxification against superoxide radicals. Although all *Lb. sakei* strains harbor a *katA* gene **(Najjari et** *al* **., 2008).** The level of resistance to various oxidative stresses is strain-dependent **(Guilbaud et** *al* **.,2012)**. Among Lactobacilli, *Lb. sakei* is one of the best equipped to cope with growth under aerobiosis, a condition that does not lead to improved growth yield, but procures a better tolerance to oxidative stress **(Zotta et** *al***., 2017).** In addition, in *Lb. sakei* mixed cultures, the ability to survive various oxidative stresses also depends on the composition of bacterial population. Indeed, in co-cultures with three different strains, it was shown that some strains may have different behavior depending on the other strains present in the mixtures. As well, some strains may behave as helper or burden to their companions in mixed cultures **(Guilbaud et** *al***.,2012)**. Resistance to high salt concentrations that can occur in fermented meats and to the cold temperatures that are of current use for the storage of raw meat products has also been documented. The optimal *Lb. sakei* growth temperature is 30 °C. Both growth rate and final cell density reached after exponential growth decrease when incubation temperature is lowered. A slow growth is still observed at 4 °C **(Marceau et** *al* **.,2003)**. The combination of low temperature and high NaCl concentration drastically impairs *Lb. sakei* growth but acts synergistically and prolongs survival of the cells during stationary phase **(Marceau et** *al* **., 2003)**. The ability to cope with high NaCl concentration, acidic pHs, or high temperatures has been shown to vary between the strains and to involve the expression of regulators from the two-component system family **(Bonomo et** *al* **., 2010)**.

II.4. Intra-species diversity of *Lb. sakei*:

II.4.1. The *Lb. sakei* Clade:

Lb. sakei has been described as closely related to *Lactobacillus curvatus*, *Lactobacillus fuchuensis*, and *Lactobacillus graminis* **(Pot et** *al***., 2014)**. These psychrotrophic species are known to be adapted to the meat environment, i.e., to the low temperature of storage used for meat products. The existence of this clade was confirmed through the comparison of the genome sequences available for several strains belonging to these species **(Zheng et** *al***., 2015)**. These comparative analyses also revealed that the closest species to this group was *Lactobacillus selangorensis* initially isolated from Malaysian food **(Leisner et** *al***., 1999)**. To illustrate this, we have drawn a phylogenetic tree (figure4) made from the *rpoB* gene sequence alignment, a method that has been reported as accurate for closely related species and sub-species **(Adékambi et** *al* **., 2008)**.

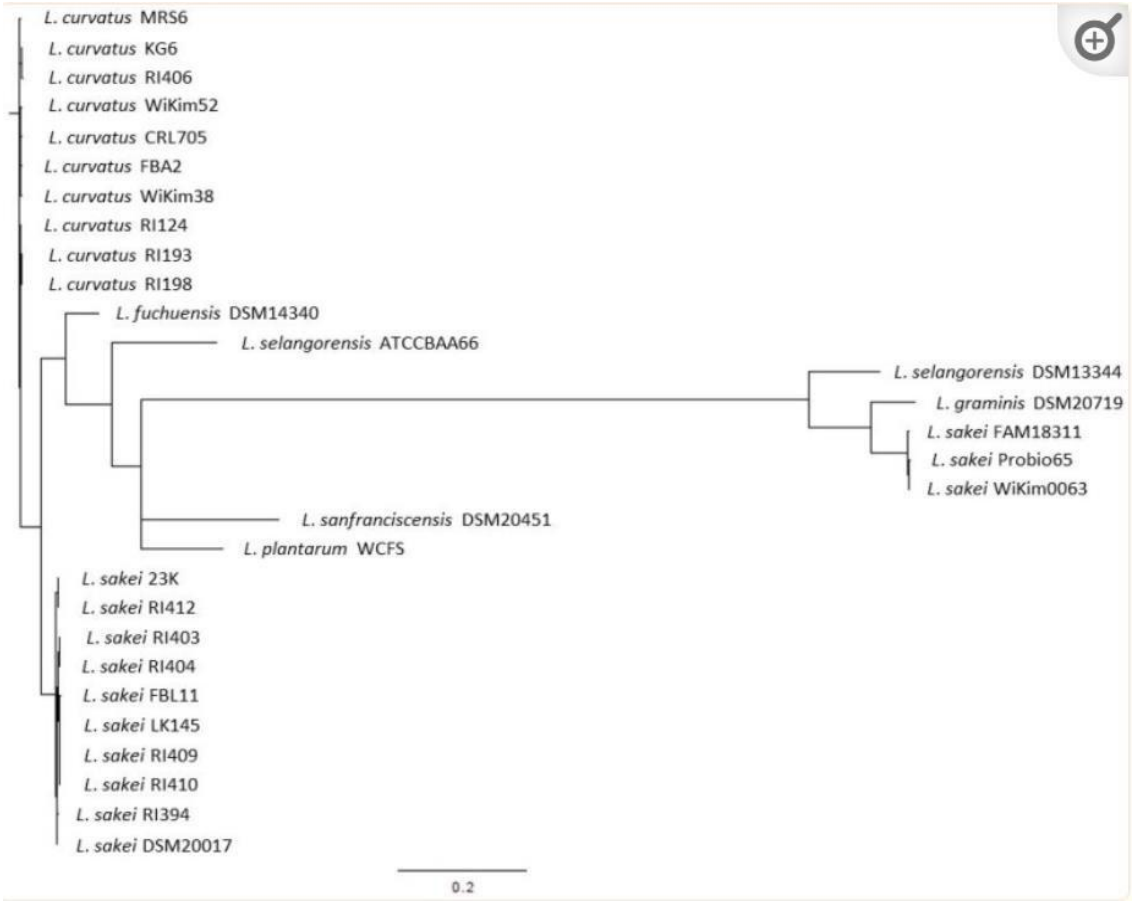


Figure 4. Phylogenetic tree drawn from the rpoB gene sequences of the main species belonging to the *Lb. sakei* clade.

Although the *Lb. sakei* species has been divided into two subspecies (*Lb. sakei* subsp. *carnosus* and *Lb. sakei* subsp. *sakei*) based on Random Amplified Polymorphic DNA (RAPD) and phenotypic characterization **(Torriani et** *al* **., 1996)**, more recent studies using the detection of strain specific genomic markers or MultiLocus Sequence Typing (MLST) revealed rather several clusters within the species **(Chaillou et** *al***.,2013)**. Such heterogeneity of the results obtained by authors aiming at clustering strains with different methods suggested a wide genomic diversity within the species.

II.5. The Genomic Diversity among *Lb. sakei* **Strains:**

(n:16) *Lb. sakei* genome sequences are publicly available **(Microbial Genomes. accessed on 17 August 2017)**, with size ranging from 1.88466 to 2.07552 Mb for the complete genomes. A strain-dependent chromosome size variation from ~1.8 to ~2.3 Mb had indeed been reported **(Nyquist et** *al***., 2011),** suggesting a large variety of strain-specific gene repertoires and therefore a wide phenotypic diversity potential of the species. The first

genome sequence of a *Lb. sakei* strain revealed the presence of genomic islands indicating putative hotspots of gene divergence **(Chaillou et *al*., 2005)**. This was confirmed in a comparative genomic analysis based on microarrays, which showed that the divergent regions may result from horizontal gene transfer **(Nyquist et *al*., 2011).** Consequently, the analysis of the diversity among a large strain collection revealed that a subset of 60 marker genes could be used as a simple PCR tool for classification of *Lb. sakei* strains into 10 clusters based on the presence or absence of these markers **(Chaillou et *al*.,2009)**. Among those genes belonging to the flexible gene pool, some may be involved in the fitness of the strains, as various transporter or cell surface component encoding genes **(Nyquist et *al*., 2011)**. Nevertheless, no link could be clearly established between the genome content of the strains and their ecological origin, whatever the method used **(Nyquist et *al*., 2011)**. Finally an MLST analysis performed on a large collection of 232 strains showed that the *Lb. sakei* species was composed of three subpopulations **(Chaillou et *al*.,2013)**.

II.6. The features of *Lactobacillus sakei***:**

II.6.1. Nutritional effect:

Fortunately, directional inoculation of these isolated functional microorganisms was instrumental in shortening the fermentation cycle, improving product quality and strengthening food flavor **(Najjari et *al*., 2020).** As common bacteria for food fermentation, LAB, which are constituted by *Lactobacillus, Leuconostoc, Lactococcus* and others, have been confirmed to be closely related to volatile flavor substances and nutrients in most types of fermented foods **(Wang et *al*., 2022).** LAB exhibit similar antioxidant capacities that can slow lipid peroxidation and prolong food shelf life **(Stecchini et *al*., 2001).** Additionally, the LAB-metabolized organic acids and bacteriocin can reduce the pH value of the system and play a so critical role in inhibiting bacteria activities that those metabolites evidently improved food safety **(Alvarez-Sieiro et *al*., 2016).** improving the production of flavor substances and shortening the fermentation period **(Bao et *al*., 2018)**. Because of its excellent performance, *Lactobacillus sakei*, a type of LAB, had been widely adopted in preparation of starter cultures **(Zhang ., et *al*., 2022)** found that L. sakei as the starter culture could tremendously improve fatty acid distribution, reshap *Lb. sakei* had the function of hydrolyzing protein and producing flavor amino acids during fermentation of meat **(Zhao *al*., 2020)**.

Lb. sakei had the function of hydrolyzing protein and producing flavor amino acids during fermentation of mandarin fish **(Zhao et *al*., 2020).** sensory properties and decline nitrite and malondialdehyde content in fermented sausages. Fundamentally, the LAB-deduced considerable changes of flavor and nutritional element derived in most case from its metabolic enzymes specificity and activity. However, the study on the activity of its metabolites was indispensable. Owning to different strain sources and survival pressures, subspecies of *Lb. sakei* widely exist in fermented foods. These subspecies adapt to the current environment through adaptive evolution, and often have better fermentation characteristics.

II.6.2. Therapeutic effect:

Lb.sakei can be used as therapeutic agents to treat various diseases: *Lb. sakei subsp. sakei* 2a, isolated from a Brazilian pork sausage sample, can inhibit the growth of *Listeria (L.) monocytogenes* in culture media and meat models **(Liserre et *al*.,2002).** *L. monocytogenes*, is a species of pathogenic bacteria that can cause a serious infection called listeriosis, it can be transmitted through food **(Adzitey et *al* .,2010).**

Lb. sakei L14 has been found to inhibit the growth of *Campylobacter (Ca.) jejuni* DC3 strain *in vitro* and in vivo in a dose-dependent manner **(John Roybert et *al*., 2022)**

Lb. sakei is a type of bacteria that has been studied for its potential immunomodulatory effects in rheumatoid arthritis (RA). *Lb. sakei RH1114 or RH1117* has been shown to suppress collagen-induced arthritis (CIA) in mice **(Jooyeon Jhun et *al* ., 2020).** Furthermore, *Lb.sakei RH1114 or RH1117* has proved immunomodulatory effect in a mouse model of rheumatoid arthritis (RA) and in human immune cells, has anti-inflammatory activity **(Sang Yun et *al*.,2015).** *Lb. sakei RH1114 or RH1117* may modulate the differentiation of T helper 17 cells and regulatory B cells **(Jooyeon et *al*., 2020)**. Arthritis is a term used to describe joint inflammation that can cause pain, stiffness, and swelling in and around the joints **(O'Neil et *al*.,2023).**

Lb.sakei has been studied for its potential to inhibit atopic eczema. Here are some key finding from the search results: *Lb. sakei KCTC 10755BP* supplementation has been shown to have a clinical effect in children with atopic eczema-dermatitis syndrome (AEDS) **(Sung-Il et *al* .,2010)** *Lb. sakei probio 65*, isolated from *Kimchi*, has been found to have inhibitory effects on atopic dermatitis-like skin lesions, emollients containing *Lactobacillus* have been

found to decrease interleukin 4 and immunoglobulin E levels and inhibit atopic dermatitis symptoms. **(J-Y Kim et *al*., 2013).** *Lb.sakei CVL-001* has been studied for its potential to improve the recovery of dextran sulfate-induced colitis in mice. *Lb. sakei K040706* has been found to have an anti-colitis effect via suppression of inflammatory responses in the dextran sulfate sodium-induced colitis mice model **(Seunghwan et *al*., 2017)**. Oral administration of *Lb.sakei CVL-001* has been found to improve recovery from dextran sulfate-induced colitis in mice by microbiota modulation **(Dong-Yeon et *al* ., 2023).**

Lb. sakei RH1114 or RH1117 has been found to have anti-inflammatory activity. And regulate immune cell differentiation in animal models of rheumatoid arthritis **(Jooyeon et *al* .,2020).** the immunomodulatory effect of *Lb.sakei RH1114 or RH1117* in a mouse model of rheumatoid arthritis (RA) and in human immune cells (**Sang-Jun et *al*., 2020).** The strain OK67 ameliorates collagen-induced arthritis in mice by inhibiting NF-κB activation and restoring Th17/Treg cell balance Author links open overlay by **(Sang-Yun et *al*. 2015)** While these findings suggest that some studied strains of *Lb.sakei* may have potential as an immunomodulatory and anti-inflammatory agent.

There is currently limited research on the effect of *Lb. sakei* on COVID-19 specifically. However, there is some research on the potential immunomodulatory and anti-inflamatory effects of *Lb.sakei*, which may have implications for COVID-19.

The therapeutic efficacy of the strain *Lb. sakei* Probio65 which is isolated from Kimchi, a type of fermented cabbage from Korea for COVID-19 through its inhibition of viral multiplication and of the subsequent pathogenicity. These findings support its potential as a microbiome drug that might be used to prevent severe COVID-19 infection and to shorten the duration of infectiousness; it may therefore be useful as an adjuvant along with the currently available treatments. The Inhibition of SARS-CoV-2 and the Modulation of Inflammatory Responses by the strain Probio65 Extract **(Irfan et *al* ., 2022)**.

II.6.3. Biotechnology features for dry fermented meat products:

II.6.3.1. Safty of starter culture:

The starter culture should grow well under the physical and chemical conditions of the sausages and be harmless to humans. To ensure the safety of starter cultures, hemolysis, toxin production, and biogenic amine production by the microorganisms in the starter culture must be evaluated **(Sirini et *al*., 2022).** Some starter cultures produce biogenic amines in fermented sausages, and thus, starter cultures producing no biogenic amines need to be

developed, and processing methods such as packaging, additives, hydrostatic pressure, and smoking can also reduce biogenic amines **(Özogul et *al* ., 2018).**

II.6.3.2. Maintining the color:

Starter culture plays a role in maintaining the color of fermented sausages by producing nitrate reductase and nitrite reductase, which reduce nitrate and nitrite, respectively **(Löfblom et *al*.,2017)**.

II.6.3.3. Enzyme activities:

During the ripening of fermented sausages, enzyme activities derived from starter culture can positively affect the sensual elements of the fermented sausages **(Wang et *al*., 2019).**Thus, the microorganisms of the starter culture should be evaluated, for degradation of carbohydrates, proteins, peptides, and lipids to produce a pleasant aroma and taste in fermented sausage.

II.6.3.4. *Lb.sakei* as a starter and functional starter:

Lb. sakei highly adapted to grow in meat environment in which it can outcompete undesired microorganisms, including pathogenic species **(Chaillou et *al*., 2013).** For this reason , it is often responsible for natural fermentation of dry fermented sausages **(Hugas et *al* ., 1993).** Because of this aptitude, selected strains of this species are widely used as starter cultures in meat fermentation, they have an important biotechnological effect in the fermentation of food products such as meat and vegetables. They have also been studied for their probiotic potential and their ability to produce anti-*Listeria* bacteriocin. Previous studies have shown that some strains may produce three different antimicrobial compounds: sakacin P, a 6.8 kDa molecule identical to the 30S ribosomal protein S21 of *Lb. sakei subsp. sakei* 23 K and a third 9.5 k Damolecule identified as a histone-like DNA-binding protein HV **(Carvalho et *al*.,2010).** *Lb.sakei* have large genetic equipment for resisting harsh conditions encountered during meat storage and meat processing. The species have resistance to oxidative stress and high salt concentrations, also have resistance to the cold and low temperatures and acidic pHs **(Zagorec et *al*., 2017)**

-The use of *Lb. sakei* as Protective Culture:

Besides its utilization as a starter, mainly based on acidification capacities of the species, the evidenced antibacterial, bacteriostatic, and bactericidal properties of *Lb. sakei* have prompted researchers and industrials to explore and develop such biopreservation properties in fermented meat and in fresh meat products. Two main aspects have been considered and studied: the bacteriocinogenic activities that can be effective in fermented or fresh meats and

the biopreservation action relying on this property and on competitiveness allowing biopreservation of fresh meat. Both pathogenic and spoilage bacterial species have been considered as targets for biopreservation in order to increase safety and shelf life of the products. The most largely explored area has first been the bacteriocin production abilities and their activities against pathogens. Many studies have reported bacteriocin production by different *Lb. sakei* strains and in some cases the molecular mechanisms have been elucidated **(Castellano et** *al* **., 2005)**.

The ability of protective cultures of interest to develop in fresh meat during their conditioning at low temperature has also been explored through their protein hydrolysis and amino acid production capacities **(Castellano et** *al* **., 2012)**. However, although these reports have deeply characterized the mechanisms of bacteriocin production even at genetic level, the effectiveness of bacteriocin-producing strains and *Lb. sakei* in food products and particularly in meat appeared to be limited biopreservation trials in fresh meat products have been directed either against pathogens, the main emblematic ones in meat being *L. monocytogenes* and *Escherichia (E.) coli*, or against spoilers aiming at extending shelf life.

The strategy generally relied on the selection of candidate strains from the targeted products. From sliced cooked ham artificially contaminated with *L. monocytogenes*, indigenous *Lb. sakei* strains were isolated for their ability to limit the development of this pathogen. These *Lb. sakei* strains revealed to be able to limit the development of *L. monocytogenes* and *E. coli* O157:H7 **(Bredholt et** *al* **., 1999)**. These selected strains have been used successfully as protective cultures with a good consumer acceptance of the so protected hams **(Bredholt et** *al* **., 2001)**. Similarly, Vermeiren and coworkers developed strategies in order to set up protective cultures for enhancing cooked ham shelf life and hygienic quality in the 2000s **(Vermeiren et** *al* **., 2006)** .

They selected potential candidates issued from a large screening of meat products and performed co-cultures with such a selected *Lb. sakei* strain and a bacteriocin-producing strain in a model cooked ham and showed that the bacteriocin producer was inefficient against the spoilage organisms tested (*Leuconostoc (Lc.) mesenteroides* and *Brochothrix (Br.) thermosphacta*), whereas the selected strain was able to lower their growth. Fresh-chilled lamb and vacuum-packed beef have also been screened in order to select putative protective cultures **(Jones et** *al* **., 2010)**.

First, LAB isolated from these products were tested in in vitro models for their ability to limit the growth of selected pathogenic or spoilage bacteria. Six isolates (five *Lb. sakei* and one *Lactococcus (La.) lactis*) appeared to exhibit inhibitory properties either against *Br. thermosphacta*, *L. monocytogenes*, or *Clostridium (Cl.) estherteticum* **(Jones et** *al* **., 2008)**. These selected strains were then successfully tested after inoculation in lamb or beef during prolonged storage at low temperature under vacuum **(Jones et** *al* **., 2009)**.

The best preservation was achieved by using a cocktail of three of these *Lb. sakei* strains **(Jones et** *al* **., 2010)**. Such a strategy, relying on strain cocktail development, has later revealed to be promising with *Lb. sakei* as a protective species. Indeed, in 2014, **Chaillou et** *al***., 2014)**. also tested three different *Lb. sakei* strain cocktails in order to evaluate their ability to inhibit the development of the pathogens *Salmonella (Sl.)* typhimurium and *E. coli* O 157:H7 and the spoiling species *Br. thermosphacta* in ground beef **(Chaillou et** *al* **., 2014)**. One of these cocktails was revealed to be efficient by limiting the development of both *Sl.* Typhimurium and *E. coli*. In cured products such as bacon, *Lb. sakei* has been reported to be an efficient protective culture, able to limit the spoilage due to the development of *Lc. mesenteroides* **(Comi et** *al* **., 2016)**.

These approaches appear promising for future development of biopreservation for enhanced shelf life and safety of meat products, once the global ecosystems of such products and the complex equilibrium driving their evolution during storage at low temperature are assessed. Yeasts and molds are not among the most important microorganisms responsible for spoilage of meat products, but they may be of concern in other food products such as dairy products, cereals, fruits, or vegetables. The antifungal activity of *Lb. sakei* isolates from kimchi has been studied. A strain active against *Penicillium (P.) brevicompactum* through the production of organic acids has been reported **(Huh et** *al* **., 2016)** . *Lb. sakei* was shown to inhibit the growth of *Colletotrichum (Co.) gloeosporioides*, *Botrytis(Bo.) cinerea*, *P. expansum*, and *Aspergillus (A.) flavus*, but the inhibiting molecule(s) were not clearly identified (**Cortés-Zavaleta et** *al* **., 2014)**.

II.6.4. The use of *Lb. sakei* as a Starter for fermented sausage:

As mentioned above, the species *Lb. sakei* exhibits properties that ensure it a high fitness for meat environments, especially for fermented ones. Belonging to the indigenous microbiota of meat, it is spontaneously active for the fermentation of artisan sausage. In association with coagulase negative *Staphylococci*, it has been used as a commercial starter for many years

for sausage making in different countries worldwide and is especially important in Western Europe **(Talon et** *al* **., 2008)**. A large biodiversity of strains has been reported, and numerous studies have described strain isolation from fermented meat products. The aptitude of starter strains to fast acidification is an important characteristic as it has an impact on taste and safety, aroma, and bacteriostatic and bactericidal effects **(Leroy et** *al* **., 2006)**. Depending on raw materials and indigenous microbial communities, a wide variety of products exist. Besides this main importance in Western Europe, it is noteworthy that *Lb. sakei* is also involved in fermented meat products in Asia **(Tamang et** *al* **., 2016)** , as name chua in Vietnam **(Nguyen et** *al* **.,2010)**, or *Mum*, a traditional meat-fermented sausage, in Thailand **(Wanangkarn et** *al***., 2014)**. *Lb. sakei* has been also identified in adventitious llama meat fermentation made in the Andean region **(Fontana et** *al***., 2016).** Along with their use as starters in association with *Staphylococci* for sausage making, the interactions between these two species have been considered. It has been shown that some of these coagulase negative *Staphylococci* could affect both growth and proteolytic activities of *Lb. sakei* strains. Such interactions are therefore of interest and should be considered for selection of starters in order to improve the organoleptic properties of fermented sausages **(Tremonte et** *al* **., 2010)**.Besides the role of the species in fermentation, *Lb. sakei* strains isolated from fermented products have also been tested for their health benefits or for their potential use as probiotics to be added in food **(Lee et** *al***., 2016)**. Resistance to conditions encountered in the gastrointestinal tract (bile salt, pepsin, and acidic pH) was observed as well as the ability to adhere to intestinal cells **(Song et** *al***., 2015).** Putative health benefits of *L. sakei* as immunomodulatory activity has been reported **(Lee et** *al* **.,2016)**. A positive effect of extracts of brown rice fermented with *Lb. sakei* on bone formation has also been reported **(Kang et** *al***., 2014)** . However, most of the studies dealing with probiotic or health effect were performed *in vitro* or using cell cultures or mice models. Water content and pH in fermented sausages decrease during fermentation, and several biochemical reactions, including lipolysis, occur **(Fernández et** *al***., 2000).** Lipids in fermented sausages can be oxidized to free fatty acids **(Chen et** *al***., 2017),** enhancing the flavor of fermented sausages **(Flores et** *al* **., 2011).** LAB, yeasts and molds are known to hydrolyze triglycerides and increase the free fatty acids content in fermented sausages **(Chen et** *al***., 2017).** Subsequently, free fatty acids undergo enzymatic and non-enzymatic oxidation, yielding alcohol, aldehydes, carboxylic acids, and other flavor compounds **(Casaburi et** *al***., 2007).** Thus, applying microorganisms with high lipolytic activity to the manufacturing process

might improve the sensory quality and of fermented sausages **(Casaburi et *al*., 2007).** When fermented sausages were inoculated with *Lb. sakei* and combinations of different *Staphylococcus* species, hexanal significantly decreased with the addition of starter cultures, reflecting decreased rancid flavor in the fermented sausages **(Fonseca et *al*., 2013).**

Lb.sakei influences colour, flavour and texture of sausage during meat processing **(Najjari et *al* 2016)**:

here are some ways in wich *Lb. sakei* can influence the color, flavor, and texture of sausage during meat processing **(Najjari et *al* 2016)**:

Color : inoculation of *Lb.sakei* can improve the red color of dry fermented sausages. This is because the bacteria produce nitric oxide, which reacts with myoglobin in the meat to form a stable red pigment .

Flavor : *Lb.sakei* can contribute to the development of flavor in fermented sausages . The bacteria produce lactic acid, which can lower the pH of the sausage and create a tangy flavor **(Jose et *al*., 2018).** Additionally, *Lb.sakei* can produce other flavor compounds, such as diacetyl and acetaldehyde.

Texture : Inoculation of *Lb.sakei* does not appear to have a significant influence on the texture of fermented sausages **(Ammara et *al* .,2021)**

However, other studies have shown that addition of other lactic acid bacteria, such as Lb. plantarum, can improve the texture of reduced-salt dry fermented sausages **.** Overall, the use of *Lb.sakei* in meat processing can have a positive impact on the quality of dry fermented sausages, particularly in terms of color and flavor **(Ji Wang et *al*., 2022)**

II.6.5. *Lb. sakei* **the evil bacteria :**

It should be also noticed that besides the *arc* operon responsible for arginine utilization through the arginine deiminase pathway, some strains harbor a second operon encoding the enzymes of an agmatine deiminase pathway **(Rimaux et *al* ., 2012)**. The production of putrescine from agmatine has been demonstrated in these strains **(Rimaux et *al* ., 2012)**, and this may contribute to a spoilage potential of such *Lb. sakei* strains. Another concern is the carriage of antibiotic resistance genes, in particular when present in transmissible genetic elements, a property that does not fit with the necessity of starter or protective strains to be added in human food. Nevertheless, the presence of acquired genes for resistance to clinically relevant antimicrobials as tetracycline and erythromycin resistant genes has been reported in *Lb. sakei* isolated from meat or pigs **(Chang et *al* ., 2011).**

I. Material and Methods

I.1. Objective of the study:

In Algeria, the sausages are rarely consumed fermented or smoked mostly cured and cooked. In the other hand, The LAB strain *Lactobacillus.sakei subsp.sakei* BK18 isolated from laboratory-made *Kaddid* and previously selected by phynotypical-gynotypical studies for their safety criteria, appropriate technological characteristics and probiotic activity (Data not published).

The aime of this study is to formulate a recipes of dry-fermented sausage like Italian-*Salami* by the using the available row materials basically low-cost red-meat cuts and fat. After the manufacturing of sausages with controlled conditions, the count of the viable cells of *Lactobacillus.sakei* subsp *sakei* BK18 and other autochthonous/spoilage LABs during the repining will be investigated comparing to a negative control without starter. At the end of repining, the textural and sensorial parameters such as: aroma, color, taste and firmness, etc. were investigated.

The integrality of the experimental trails was carried out at the pedagogical laboratories of Biology (PLB) located at the campus of Ouzera, University of Medea during the period between May to June 2023.

I.2. Starter culture:

The strain *Lactobacillus sakei subsp sakei* BK18 stored at -25 °C in MRS broth with 25 % of glycerol were activated twice on the MRS (de Man Rogosa and Sharpe) (Conda, Spain) medium for 18h at 30 °C, then sticked on MRS agar, the purity of the colonies was checked by catalase reaction and Gram test, new stocks of pure strain were made and stored at -25 °C for future use.

I.3. Preparation of the culture for inoculation:

The strain was previously activated several times until the day of preparation of the sausages by using increased volumes of MRS broth, from 5, 10, 20, until reaching 200mL, by inoculating the medias with 0.1% (v/v) and incubated under static conditions at 30 °C for 18h. After incubation, the optic density was measured at OD_{600} (put the brand and the

country), and the concentration of cells were calculated by using growth curves of the strain previously studied (Data not published), after that, the bacterial cells are recovered by centrifugation at 6000 rpm for 5 min at 4 °C, washed twice with physiological water pH 7 and resuspended/concentrated in sterile falcon at the volume of 40 ml and stored at +4 °C until use. The count of viable cells was estimated by serial dilutions and results were expressed with CFU/mL.

I.4. Manufacturing of the Sausages:

Dry-fermented sausages used in this work **(Figure5)** were prepared by using the equipment's of local butcher in the center of Medea. The recipes dictate the composition of this meat products per kilogram that consists of 2 kg lamb meat, 786 g turkey meat 1333.2 g lamb fat, 151g sodium chloride, 66.6 g sugar, 44.4 g powder milk, 22.2 g chopped garlic, 8.8 g ground red pepper, and 13.2 g ground black pepper. All the ingredients were thoroughly mixed, and meat dough was divided into two batches: one batch was inoculated with the stater *Lb sakei.subsp sakei* BK18 concentrated in 40 ml of physiological water and the other was kept as a control system was inoculated also with 40mL of sterile physiological water. Afterwards, once the meat dough had been finished, being completely homogenized before casings were stuffed. Meat dough was packed and shaped into natural casings (sheep gut) and transported to the laboratory for drying in ventilated oven (Memmert, Germany). The concentration of the starter was estimated at ~4.85×10^5 colony forming units per gram (CFU/g). The unfermented products were placed in a ripening for 15 days. During the first seven days—fermentation period—temperature was kept at 20–22°C and relative humidity (RH) oscillated to 90–95%. The next 8 days—ripening period—temperature was kept at 24°C and RH range was 80–85%. From each batch, samples were used for enumeration of LAB and total mesophilic flora at initial day (d_0), d_3, d_7, d_{10}, and d_{15}, the sensory analysis was performed after 15 days of drying and repining.

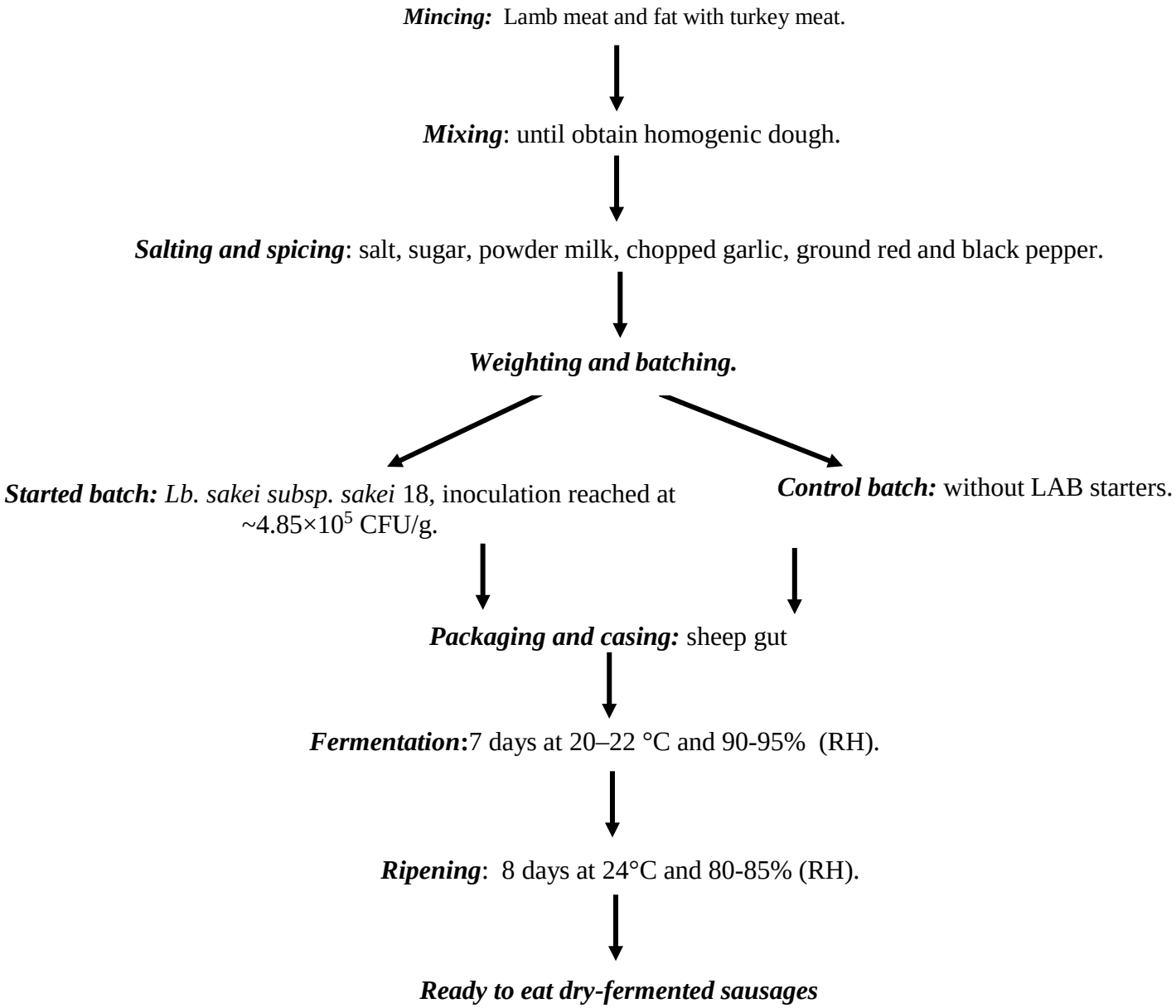


Figure 5. Diagram of dry-fermented sausages preparation.

I.5. Microbiological Analyses:

Sausage samples were ground in a sterilized mortar and transferred (10g) into a sterile stomacher bag and 90ml of tryptone salt-diluent (tryptone ,1g /L ;Nacl 0.85g/L ;Tween 80 1ml/L) was added after homogenization in a stomacher (400 Circulator , Seward , UK) for 3 min at 250 rpm **(Boubakri et *al*., 2022)**, the suspension was left at laboratory

temperature for 15 min for bacterial revivification. From this stock solution, dilutions were prepared, and count of each microbial group was made in duplicate by using the adequate dilutions. Total mesophilic flora (TMF) was determined by using plate-count-agar (PCA) (**Conda, Spain**) incubated at 30°C during 72 h in aerobic conditions, and lenticular colonies that have grown on masse were counted. Furthermore, the count of LAB was performed on MRS agars (pH6.2) and incubated aerobically at 30 °C during 72h **(Boubakri et *al*., 2022)**. Negative controls were made with sterile broth and media.

The enumeration of the different floras was carried out using a colony counter, taking into account that the plates contain between 30 and 300 colonies. The number per gram of sausage is calculated using the following formula **(ISO 7667 June 1984 V 08-001):**

$$\mathbf{N} = \frac{\mathbf{\Sigma_c}}{(\mathbf{n1} + \mathbf{0.1.n2})d}$$

$\sum$ c: The sum of the colonies counted on all the plates retained ;

n1: the number of cans retained for the first dilution ;

n2: the number of cans retained at the second dilution ;

d: is the dilution ratio corresponding to the first dilution.

If the two petri plates contain less than 15 colonies, the arithmetic mean *y* of the colonies counted on two plates, and the results are expressed in microorganism by gram as follows:

$$NE=y/d$$

d: the dilution ratio of the parent suspension.

I.6. Sensory analysis:

Sensory evaluation was performed for the two batches of sausages at the end of the maturation process. Total of n: 30 panel members were selected, 12 females and 17 males gender, including graduate students, teaching profession and other professions at Medea University, and also some dietetic experts who have experience in the consumption of fermented products. A preparatory session was held before the test so that a member could thoroughly discuss and understand each feature of the sausages to be evaluated (two pieces per person) with a thickness of 1.5 cm cut with a numerically determined sharp knife and coded with numbers and served immediately on a white glass plate covered with plastic wrap each piece was served 3 times, water and cut the chocolate were used after ticking each piece to cleanse the palate between samples. A quantitative descriptive analysis was

used to assess differences and a structured 5-Points pleasure-scale was used (1= highly disliked, 2= slightly disliked, 3=average ,4=like, 5 =Very like) to record the sensory attributes of different school batches **(Mina et *al*., 2014).** The panel members can filfull theirs choices by using *Google-Form* and the data was collected for analyzing and processing.

I.7. Statistical analysis:

Agar assays were performed by duplicate and growth curves by triplicate. The data was statistically analyzed by using Microsoft Excel (2016), and the results were expressed as: SM± SE, where standard mean (SM) and standard error (SE) were calculated. For sensory analysis the means and standard deviation (SD) were calculated and expressed as: SM ± SD. The differences were considered as significant at $P \geq 0.05$ value. The results were presented by curves for the count of LAB and TMF, and for the sensory results were presented by radar graph using Microsoft Excel (2016).

I.8. Financial support:

A major part of the trails was performed by using the equipment of PLB further more MRS and PCA media were purchased by PLB. The row ingredients for manufacturing of sausages were bought by the students. The plastic material such as petri-dishes and tips were purchased by the framer of this work.

Results and Discussion

III. Results and Discussion

The aim of this study is to study the viability and the sensory effect of the selected strain *Lb. sakei subsp sakei*BK18 during the repining of *Salami*-type dry-fermented sausages by using the available row materials.



Figure 6. The steps of preparation sausages.
[a]Mixing the ingredients and batching; [b]stuffing the mixture inside the intestine
[c]formation of sausage kernels; [d]hanging of sausage inside the oven for repining
[e]Dried -fermented sausage ready to eat.

III.1. Macroscopic and microscopic aspect of *Lb. sakei subsp sakei* **BK18:**

The macroscopic observation of *Lb. sakei subsp sakei*BK18 in MRS agar show a dense (colonies, sometimes circular, small to medium-sized, about 1 mm in diameter, whitish or milky, with a regular and smooth periphery. Most of LABs are catalase negative or pseudo-catalase.

a b

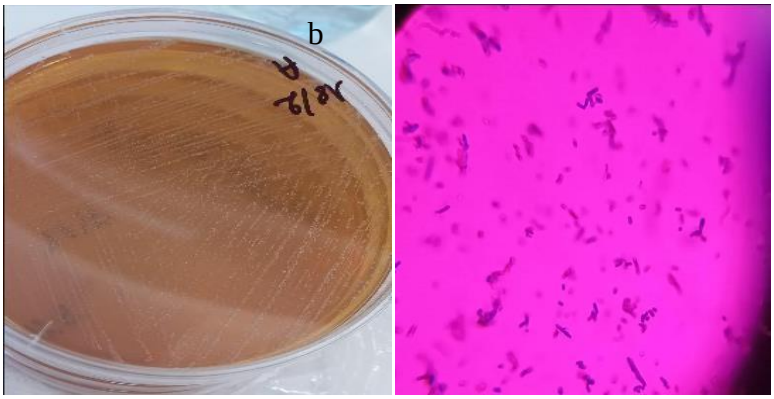


Figure 6. Macroscopic and microscopic aspect of *Lb. sakei subsp sakei*BK18
(a)colonial aspect on MRS agar ;**(b)**microscopic observation

III.2. Evaluation of the microbiological changes during the repining:

III.2.1. Enumeration of total aerobic mesophilic flora (AMF) and LAB:

The results of the enumeration of AMF and the LAB were illustrated by the graphs. The initial load of AMF (1.845×10^5 CFU/g) while the lactic load is (3.65×10^3 CFU/g). After 3 days of drying of the sausages, the two batches show a decrease in the load in aerobic mesophilic which is more significant in the control sausages, it can be due to the combined effect of salting/drying on the endogenous flora, at the same time the load in lactic acid flora presents a progression in the inoculated sausages (2.918×10^8 CFU/g) of the inoculated sausages and 5.204×10^3 CFU/g for the control. After 7 days of drying, the LAB load continues its progression, it is 1.945×10^7 CFU/g in the inoculated sausages and 2.981×10^3 CFU/g in the control sausages, which indicates that the strain *Lb. sakei subsp sakei* BK18 has adapted well to hostile conditions, and it shows a high growth rate during the first few days from drying.

These results agree with those of **Lorenzo et *al*., (2014)** concerning the ability of survival of *Lb. sakei* strains inoculated in sausages during repining/drying period. Same results were observed by other authors **(Zhao et *al*., 2011; Essid and Hassouna., 2013).**

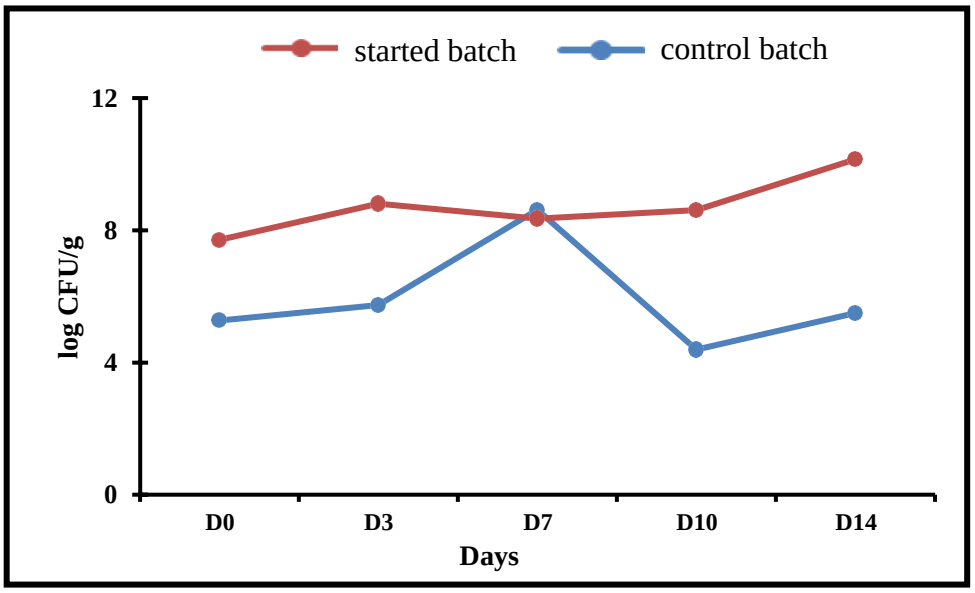


Figure 7. Evolution of LAB during the ripening of sausages.

This graph illustrates the variations of LAB load (expressed as log CFU/g) within sausages over a duration of days. The blue line corresponds to sausages without a starter culture, while the red line represents sausages with a starter culture.In both batches,a gradual increase of LAB load is observed.from day0 to day3,the sausages without a starter culture exhibited a specific range of values between3,7 and 4 log CFU/g. On the other hand,the sausages with a starter culture displayed a range of values between 8,4 and 9 log CFU/g. Subsequently, a slight reduction in load occurred until day 7, with the sausages without a starter culture reaching a range of values between 4and 3,7 log CFU/g, While the sausages with a starter culture decreased to range of values between 9 and 7,5 log CFU/g. However, from day 7 to day 14, there was a significant increase in load, reaching a maximum threshold value of 5log CFU/g. Furthermore, a notable increase in load was observed in sausages with a starter culture,peaking at 12log CFU/g. This significant increase can be attributed to the presence of the bacteria inoculated from the starter culture, specifically*Lactobacillus sakei subsp sakei* BK18, Which facilitated this growth.This outcome is in agreement with those reported by other authors **(Baka et *al*., 2011; Essid and Hassouna., 2013; Zhao et *al*., 2011; Lorenzoet *al*., 2014)** who displayed that the use of selective starter to produce sausages affect significantly the amounts of these microbiota.

In the other hand,when the sausage were inoculated with starter culture values of (TAMF) were recorded starting from day 0 to day 14 , and this results are showin in the following curve (Fig 7).

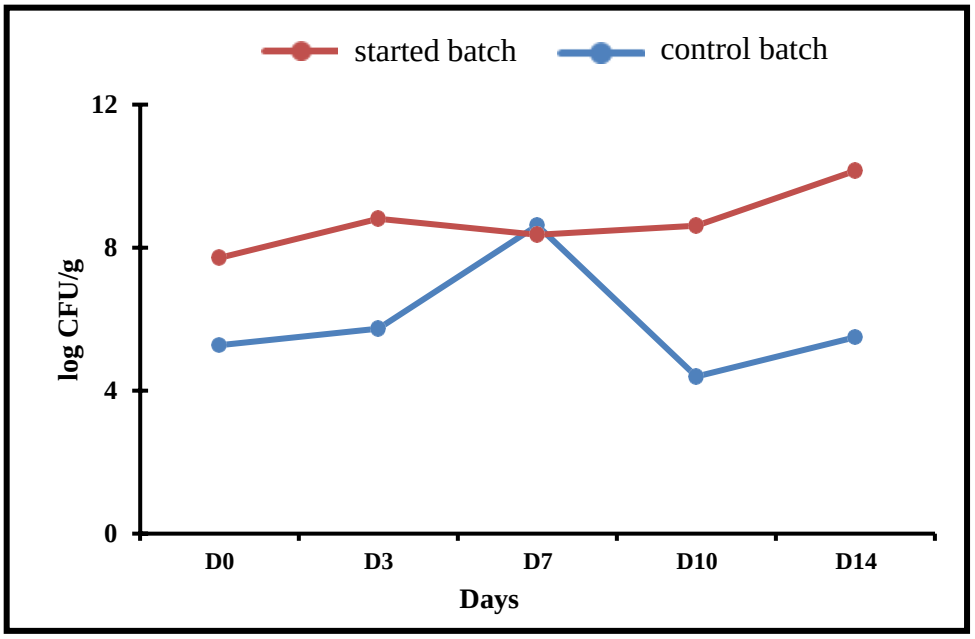


Figure 8. Evolutionof total aerobic mesophilic flora (TAMF) during the repining of sausages .

This graph depicts the variations in the proportion of aerobic mesophilic flora (expressed as log CFU/g) within sausages over a period of days.The blue line represents sausages that were not supplemented with a styarter culture, whereas the red line represent sausages that were supplemented with starter culture.We observed an initial rise the load of aerobic mesophilic flora from 4.5 log CFU/g (day 0) to a peak of 8 log CFU/g on the 7th day, followed by a slight decline on day 10 to reach 4log CFU/g. Subsequently, there was a gradual increase to reach 4.5 log CFU/g.

In the case of sausages supplemented with a starter culture, there was an initial increase in the (TAMF) load from 7.8 log CFU/g (day0) to 9 log CFU/g on day 3, followed by a slight decrease on day 7 to reach 8 log CFU/g.Thereafter, the load gradually increased until reaching 11 log CFU/g on day14.

This increase in the (TAMF)load can be attributed to viable counts, LAB at the beginning of the process could be related to the addition of starter cultures, specifically *Lb. sakei*

*subsp sakei*BK18. This result is in concordance with those of **Casaburi et *al*., (2008); Casquete et*al*., (2012); Ruiz-Moyano et*al*., (2011); Zaho et *al*., (2011)** who showed that the use of selective starter to produce sausages affect significantly the amounts of these microflora and the introduction of the starter culture led to the increase in total bacterial populations. The concordance between LAB and TAMF counting (fig 6 and 7) suggest that a part of LAB and *Lb. sakei subsp sakei* BK18 could grew in both media. Furthermore, the high counting of LAB and TAMF in the started batch comparing to the control batch evidencing the high adaptation and viability of *Lb. sakei subsp sakei* BK18 during the drying/repining.

III.3. Sensory evaluation:

The results of a public opinion about testing the dry-fermented sausage are represented by the radar shown in the figure 8. One scale evaluation from 1 to 5 were used to describe the product and make preferences between started batch and control batch.

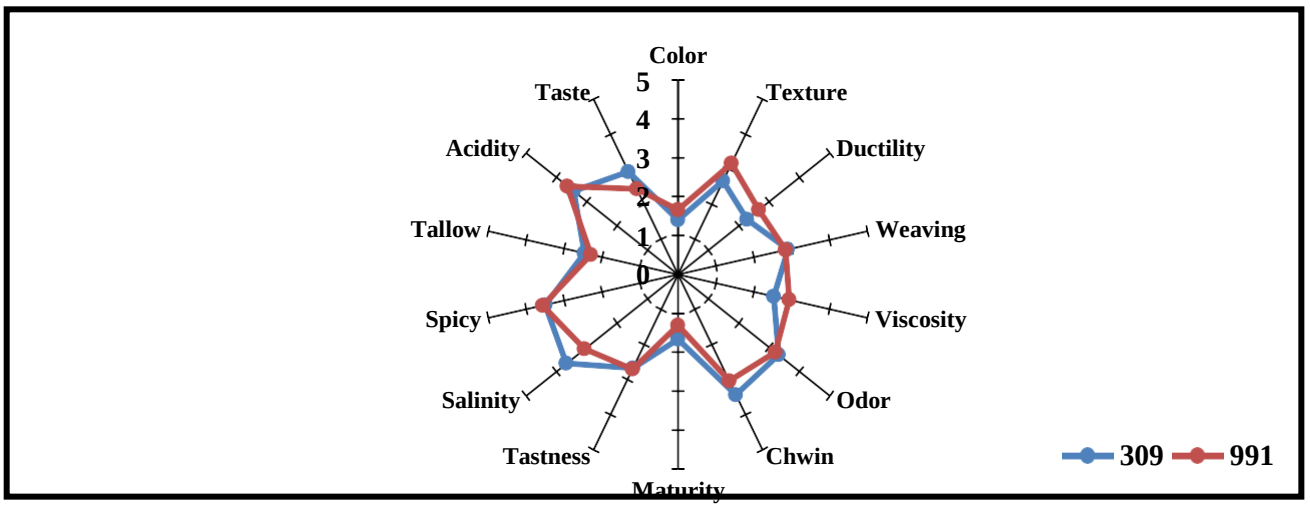


Figure 9. Comparative Sensory-evaluation between starter-inoculated and control sausages.

309: Control batch; 991: Started batch

Regarding the color evaluation, we have noticed that most of the tasters answers for control batch were for *very dark* color corresponding to score of (1,40 ± 0,24), in the meanwhile for started batch, most of the answers were between *very dark* and *dark* respectively and the mean value was (1,67 ± 0,30), this significative difference between batches could be related to the slight change in color tone due to the effect of the strain

Lb.sakei subspsakei BK18 in inoculated batch, which might had an effect of lightening of the color tone from *very dark* to *dark*. These results agree with **Ammara et** *al***., (2021)** in which they found that *Lb. sakei* KCTC-5053 influenced the red color, due to the nitrate-reductase-activity known for some LABs associated with fermented meat products and the production of nitrosyl-myoglobin responsible for the *red-color* by the reaction of nitrogene monoxide with myoglobin.

Most of the testers prefer the texture of the started batch scored with 3,10 ± 0,13 corresponding to *medium*, while the control batch had a score of 2,67 ± 0,25 between *bush-medium*, those results agree with the writers **Yingying et** *al***., (2022)** in which they found that the inoculation with *Lb. curvatus* and *Lb. plantarum* had a positive impact on the texture attributes of the reduced-salt sausages.

Regarding the ductility, the tasters describe the control batch as *solid=2* close to score of 2.27 ± 0.19), while about started batch, most of the choices were pretty close *medium=3* corresponding the value of 2.80 ± 0.22, those results could be explain by the proteolysis effect of *Lb.sakei subsp.sakei* BK18 on sarcoplasmic Lamb-proteines (data not publisded) The proteolyltic enzymes hydrolyse meat proteins during the fermentation, which can contribute to increased ductility evidenced by some studies such as **Ammara et** *al***., (2021)** by using the strain *Lb. sakei* KCTC-5053 and **Najjari et** *al***., (2020)** by using the strain *Lb.sakei* BMG 95.

Concerning the acidity, it was proved *in vitro* that the starter strain *Lb.sakei susp sakei* BK14 has a quick and hug acidification of meat-broth medium and MRS-broth even with 10% of NaCl, but according to the testers the started batch is slightly *acid* to *sour* than the control close to *medium*. Those results could be explained the application *in situ* of BK18 in the sausage-system had a differences physicochemical condition specially water activity (a_W) than broth media. Furthermore, the proteolytic activity of LAB could produce small peptide and free amino acids which can buffer the system, which also led to softening and increasing ductility as explained **Najjari et** *al***. (2020) Ammara et** *al***. (2021**). *Lb.sakei* species plays a crucial role in the fermentation process by producing lactic acid. This acidification process lowers the pH of the sausage, creating an environment that inhibits the growth of spoilage microorganisms. The acidic conditions and pH control provided by

Lactobacillus sakei contribute to the preservation of the sausage and help maintain its characteristic aroma.

For weaving most of the testers don't make difference between the two batch and they give the same score of 2.90±0.20 corresponding to *coherent* which means that the starter didn't affect these textural attribute, similar results were observed by **Yingying et** *al***., (2022).**

Concerning the odor, most of the tasters describe the control batch as a *little foul* corresponding to score mean of 3.40±0.31 In the other hand the started batch with BK18 was scored with 3.40± 0.29 corresponding of the choice *beautiful*. It's clear that the strain BK18 has a significative enhancement on the odor of the dry-fermented sausages. Similar results were observed for the application *Lb. sakei* 23K isolated from French sausages *Saucisses sec* (**Najjari et** *al***., 2020)**, and *Lb. sakei* BMG 95 isolated from anchovies **(Chaillou et** *al***., 2005)**, and *Lb. sakei* BMG 37 isolated from sheep meat **(Najjar et** *al***., 2008)**. The same writers were found that *Lb.sakei* species produce various volatile compounds during the fermentation process, which can contribute to the overall aroma of the sausage. These compounds include aldehydes, alcohols, esters, and sulfur compounds. Each of these compounds has a distinct odor profile and can contribute to the characteristic aroma of fermented sausages. *Lb.sakei* possesses amino acid decarboxylase enzymes that can convert amino acids into biogenic amines, such as histamine, tyramine, and putrescine. Biogenic amines are known to have a strong impact on the odor and flavor of fermented products. The production of specific biogenic amines by *Lb. sakei* strains can influence the aroma profile of the sausage.The used of mixed starters cultures, such as the combination of *Lb.sakei* and *Staphylococcus xylosus* strains, the interactions between different bacterial species can influence the aroma development of fermented sausages. Some strains of *Lb. sakei* are known to inhibit the growth of undesirable bacteria, including those that may produce off-odors. By outcompeting or suppressing the growth of these bacteria. Antioxidant activity of *Lb.sakei* species has been found to exhibit antioxidant properties, primarily attributed to the production of various compounds such as organic acids, peptides, and bacteriocins. These antioxidant compounds can scavenge reactive oxygen species (ROS) and inhibit lipid oxidation, thereby reducing the generation of volatile compounds that contribute to off-odors in fermented sausages **(Irene et** *al***., 2023)**.

The testers describe the sausages as *a little raw* with a slight difference between the two batches maturity. Despite, many studies were showing the effect of *Lb.sakei* on fermentation is represented by quick acidification by producing lactic acid as a metabolic during fermentation which leads to a rapid decrease in pH. The acidic environment created inhibits the growth of undesirable bacteria and promotes the growth of beneficial bacteria, thus accelerating the fermentation process. Indeed, the added sugar and sugar of meat metabolized to lactic acid. The fast sugar-metabolism by *Lb. sakei* results in a rapid decrease in available sugars, creating a selective environment that favors the growth of *Lb. sakei* and other beneficial microorganisms involved in the fermentation process. Furthermore, the competitiveness and the quick establishing of *Lb. sakei* ensures a favorable fermentation environment and helps expedite the overall process. Also *Lb. sakei* possesses various enzymes, including proteases and lipases, which are involved in the breakdown of proteins and fats, respectively. These enzymes accelerate the breakdown of complex molecules in the sausage meat, facilitating the release of nutrients and providing substrates for microbial growth. The increased enzymatic activity of *Lb. sakei* can expedite the fermentation process and enhance the overall flavor development of the sausage (**Yingli et** *al*.**, 2021).**

the tasters describe the found the started batch as less salted than the control with a score values of 3.20±0.23 and 3.67±0.28 respectively, corresponding to an *average* salinity. Salinity refers to the salt content in the sausage, which is primarily controlled by the formulation and ingredients added during sausage preparation rather than the bacteria involved in fermentation **(Ammara et** *al*.**, 2021)**

The testing panel found the started batch spicier than the control with mean scores 3.75 ±0.36 close to *spicy* and 3.60 ± 0.34 between *average* to *spicy*. According to **Ammara et** *al*.**, (2021)** *Lb.sakei* might not directly affect the spicy of sausage. Spicy refers to the spices content in the sausage, which is primarily controlled by the formulation and ingredients added during sausage preparation rather than the bacteria involved in fermentation. It's necessary to mention here that the sausage prepared don't contain sodium mono-glutamate nor nitrate of sodium, those ingredients are largely used for fermented meat products to enhance the flavor and taste.

The tallow scored at 2.40 ±0.29 for control batch and 2.30 ±0.36 those values are ranged between *rough* to *average*, this a few differences in tallow between two batches was mentioned by other studies, where didn't notice the effect of *Lb. sakei* is not typically associated directly influencing the tallow content of sausage. Tallow refers to the rendered fat from animals, particularly beef or mutton, and is not a component typically added to sausage formulations. The tallow content in sausage is primarily determined by the type and amount of fat used in the sausage preparation.

The tastiness was scored 2.70 ± 0.20 and 2.80 ±0.17 for started and control batches respectively close to *bitter* taste. Tastiness refers to the sugar content in the sausage, which is primarily controlled by the fermentation and ingredients added during sausage preparation. The sugar added during the preparation were consumed by the starters and other autochthones LABs during the fermentation of sausages. Therefore, a little difference between two batches in *tast* which described as *acceptable=3*.

Through this commensurate and the sensorial comparative evaluation, the panel testers describe the product as good quality with preferences to started batches. Most of the panel suggested improving the quality by enlarging the diameter and the size, reducing the amount of fat and improving the smell. Dried-fermented sausage are accepted by the Algerian consumer according to the results of the survey and there a possibility of eating it again as well as buying it.

Conclusions :

The use of starter cultures in food fermentations, including sausages, has allowed for a greater level of safety and quality. Undoubtedly, the addition of starter strains in the meat batter to produce sausages makes the fermentation process less prone to modifications, which are responsible for products with deviated organoleptic properties and potentially hazardous due to the presence of foodborne pathogens not inhibited during the process. However, due to the traditional and artisanal aspects that fermented sausages often possess, spontaneous fermentations are still used and represent a common practice especially in the Mediterranean countries. Isolation and selection of new strains of well-known LAB and coagulase negative cocci (CNC) species from those traditional products represents a possible alternative to the use of commercial starter cultures.

A study *in situ* was carried out comparison to a negative control, at the beginning dry fermented sausages was prepared by using of mixture containing Lamb meat and fat, Turkish meat, spices and salt, and then the sausages were hanged for fermentation and drying for 15 days in the ventilated oven under controlled conditions of temperature and relative humidity.

Based on the study conducted on the evaluation of the survival and sensory effect of the selected strain *Lactobacillus sakei subsp sakei* BK18 on dried fermented sausages prepared under controlled conditions.

According to the results the inoculated batch of sausages show an increase on the count of LAB at the end of the repining, whereas the control batch show a decrease. Those results indicate the survival capacity of BK18 and its resistance to hostile conditions of low a_W and osmotic stress. The curves of the count of TAMF at 30 ℃ were correlated with LAB curves, this indicate that major part of TAMF was LAB growing PCA agar.

The sensory evaluation of started sausages with BK18 comparing to control batch conducted through a questionnaire and public opinion poll, revealed positive results for the started batch. The sensory attributes such as aspect, color, smell, taste, hardness and acidity were perceived

as good by the participants. This suggests that the addition of *Lb. sakei subsp sakei* BK18 positively influenced the sensory characteristics of the fermented and dried sausages.

Understanding the survival and sensory effect of *Lb. sakei subsp. sakei* BK18 can aid in the selection of appropriate strain(s) for sausage fermentation, leading to improved sensory profiles and more consistent product quality. This research contributes to the broader goal of enhancing the production and sensory appeal of dry fermented sausages, meeting consumer preferences, and ensuring food safety.

The results of our research open new perspectives. To complete these studies, we propose :

- ✓ Supplement the study with the determination of aromatic profile by CPG;
- ✓ The confirmation of the survival of *Lb. sakei subsp sakei* BK18 at the end of repining by PCR.
- ✓ Enhance the shape of the sausages, the color and the flavor by adding other ingredients.

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Annex

Liquid media

Water physiology :

NaCl 9g

Distilledwater 1000mL

Autoclave at 120 C°

Tryptone-Salt Water:

Tryptone 1g

Tween801mL

Na Cl8.5 g

Distilled water 1000 mL

sterilize in autoclave at 120°C for 20 min

MRS BROTH:

Dextrose 20g

Peptone10g

Beef extract8g

Sodium acetate 5g

Yeast extracts 4g

Dipotassium phosphate 2g

Tween 801g

Ammonium citrate2g

Magnesium sulfate 0.2 g

Manganese sulfate0.05 g

Distilled water 1000 mL

pH6.2 sterilize in autoclave at 121 °C for 12 min

Gelosis MRS

Dextrose20g

Peptone10g

Beef extract8g

Sodium acetate5g

Yeast extracts 4g

Dipotassium phosphate 2g

Tween 801g

Ammonium citrate 2g

Magnesium sulfate 0.2 g

Manganese sulfate 0.05 g

Agar14g

Distilled water 1000 mL

pH 6.2 sterilize in autoclave at 121 °C for 12 min

PCA

tryptone 5g

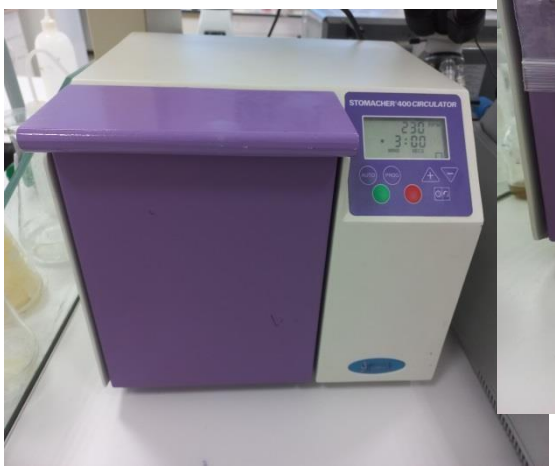
Leveure extract 2.5g

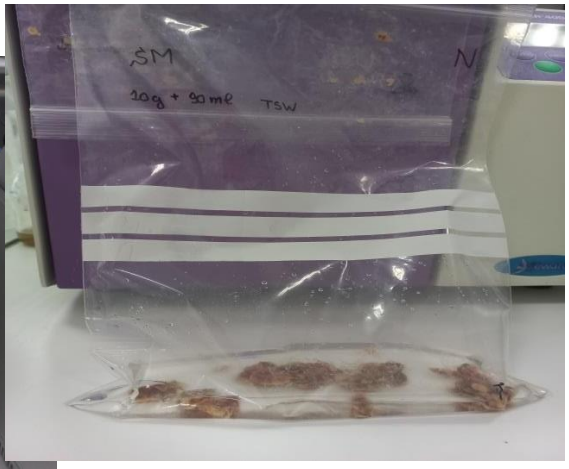
Glucose 1g

Bacteriological agar agar 12g

pH at 25 C°:7.0 ± 0.2sterilize in autoclave at 121 °C for 12 min

The equipment:





Stomacher (400 circulator) **Stomacherbags**





Oven **Micro-hound**

The food product in your hands called dry-fermented Sausage its prepared with minced lamb

meat and fat and minced turkey meat mixed with : garlic powder, black pepper, red pepper, salt,

sugar and milk powder and it's free from of chemicals additives usually used for sausages such

as nitrates sels and sodium monoglutamate, the product was prepared under controlled

conditions and its microbiological safety was proved. you have two batches of sausages

prepared with the same conditions , we would like to know your evaluation and your preferences

between the two batches

المنتج الغذائي الذي بين يديك يسمى السجق الجاف المخمر مع لحم الضأن المفروم والدهون ولحم الديك الرومي

المفروم الممزوج : مسحوق الثوم والفلفل الأسود والفلفل الأحمر والملح والسكر ومسحوق الحليب خالي من المواد الكيميائية

المضافة التي تستخدم عادة للسجق التجاري مثل النترات نيتريت الصوديوم ومونوجلوتامات، تم تحضير المنتج في ظروف

خاضعة للرقابة وتم إثبات سلامته الميكروبيولوجية. لديك دفعتين من السجق أعدتا بنفس الشروط، نود أن نعرف تفضيلك

و تفضيلك بين دفعتين.

1_What do you think about the size?

1_ very small

2_ small

3_meduim

4_big

5_very big

2_ Whatdi you think about the format?

1_unacceptable

2_I didn't like it

3_acceptable

4_I liked

5_I like it so much

3_ What do you think about the color?

1_very dark

2_ dark

3_consistent

4_ light

5_very light

4_What do you think about the texture?

1_very bush

2_bush

3_medium

4_smooth

5_very smooth

5_What do you think about the ductility?

1_very solid

2_solid

3_medium

4_flexible

5_very flexible

6_What do you think about the weaving?

1_harsh

2_a little harsh

3_coherant

4_fragile

5_very fragile

7_ How was it in viscosity?

1_very harsh

2_ harsh

3_average

4_sticky

5_very sticky

8_ What do you think about the odor?

1_very foul

2_foul

3_a little foul

4_ beautiful

5_very beautiful

9_How was it in chewing?

1_do not chew

2_ hard to chew

3_normal

4_ easy to chew

5_very easy to chew

10_What do you think about the maturity?

1_raw

2_a little raw

3_ average

4_mature

5_very mature

11_ How was it in tastiness?

1_bitter

2_a little bitter

3_average

4_sweet

5_very sweet

12_How was it in the salinity?

1_very bitter

2_bitter

3_average

4_salty

5_very salty

13_What do you think about the spicy ?

1_not spicy

2_a little spicy

3_average

4_spicy

5_very spicy

14_What do you think about the tallow?

1_harsh

2_rough

3_average

4_creamy

5_very creamy

15_What do you think about the acidity ?

1_not sour

2_a little sour

3_medium

4_sour

5_very sour

16_What do you think about the taste?

1_disgusying

2_unacceptable

3_acceptable

4_delicious

5_very delicious

17_What do you think about the quality?

1_unacceptable

2_I didn't like it

3_I liked

4_ quality product

5_High quality

18_Would you like to eat it again?

1_never

2_may be

3_very possible

4_yes, depending

5_naturally

19_ Do you add it in your diet?

1_never

2_may be

3_very possible

4_yes, depending

5_naturally

20 _ What would like to suggest enhancing the overall quality of the products?

21_ which batch number of sample do you prefer your response

23_ Other comments about what was traded above (color, smell.....Etc.)

Table. Statiscally analysis of counting lactic acid bacteria expressed by SM and SE

Jour	Dillution	Concentration	Dillution	Concentration
J0	10^{-1}	212 ± 57,98 UFC/g	10^{-5}	294 ± 11,76 UFC/g

	10^{-2}	189,5 ± 98,29 UFC/g	10^{-7}	69 ± 19,59 UFC/g
J3	10^{-1}	347 ± 43,12 UFC/g	10^{-5}	282,5 ± 20,58 UFC/g
	10^{-2}	225,5 ± 83,29 UFC/g	10^{-7}	167 ± 54,88 UFC/g
J7	10^{-1}	188 ± 60,75 UFC/g	10^{-5}	90,5 ± 26,46 UFC/g
	10^{-2}	140 ± 50,96 UFC/g	10^{-7}	123,5 ± 18,62 UFC/g
J10	10^{-3}	64 ± 7,84 UFC/g	10^{-5}	111 ± 27,44 UFC/g
	10^{-4}	49 ± 3,92 UFC/g	10^{-7}	100 ± 84,28 UFC/g
J14	10^{-3}	218,5 ± 146 UFC/g	10^{-8}	15 ± 5,88 UFC/g
	10^{-4}	300,5 ± 26,46 UFC/g	10^{-9}	102,5 ± 18,62 UFC/g

SM: Standard mean **/ SE:** Standard error

Table. Statiscally analysis of counting aerobic germs expressed by SM and SE

	309		991	
Jour	Dillution	Concentration	Dillution	Concentration
J0	10^{-1}	212 ± 57,98 UFC/g	10^{-5}	294 ± 11,76 UFC/g
	10^{-2}	189,5 ± 98,29 UFC/g	10^{-7}	69 ± 19,59 UFC/g
J3	10^{-1}	347 ± 43,12 UFC/g	10^{-5}	282,5 ± 20,58 UFC/g
	10^{-2}	225,5 ± 83,29 UFC/g	10^{-7}	167 ± 54,88 UFC/g
J7	10^{-1}	188 ± 60,75 UFC/g	10^{-5}	90,5 ± 26,46 UFC/g
	10^{-2}	140 ± 50,96 UFC/g	10^{-7}	123,5 ± 18,62 UFC/g
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J14	10^{-3}	218,5 ± 146 UFC/g	10^{-8}	15 ± 5,88 UFC/g
	10^{-4}	300,5 ± 26,46 UFC/g	10^{-9}	102,5 ± 18,62 UFC/g

SM: Standard mean **/ SE:** Standard error

Table. The sensory analysis of sausages in two batches (with and without starter) expressed by SM and SE

	309	**991**
Sensorial characteristics	**SM**± SE	**SM**± SE
SIZE	2,33 ± 0,25	2,40 ± 0,26

FORMAT	3,00 ± 0,26	2,77 ± 0,25
COLOR	1,40 ± 0,24	1,67 ± 0,30
TEXTURE	2,67 ± 0,25	3,10 ± 0,13
DUCTILITY	2,27 ± 0,19	2,80 ± 0,22
WEAVING	2,90 ± 0,20	2,83 ± 0,26
VISCOSITY	2,53 ± 0,21	2,93 ± 0,17
ODOR	3,40 ± 0,31	3,40 ± 0,29
CHEWIN	3,43 ± 0,23	3,03 ± 0,15
MATURITY	1,67 ± 0,20	1,31 ± 0,18
TASTINESS	2,80 ± 0,17	2,70 ± 0,20
SALINITY	3,67 ± 0,28	3,20 ± 0,23
SPICY	3,60 ± 0,34	3,57 ± 0,36
TALLOW	2,40 ± 0,29	2,30 ± 0,36
ACIDITY	3,43 ± 0,25	3,63 ± 0,29
TASTE	2,93 ± 0,09	2,43 ± 0,21

SM: Standard mean **/ SE:** Standard error