

Presence of bacteria in modified atmosphere packed raw chicken meat and their effect on shelf life and sensory characteristics

- A microbiological and sensory evaluation

Max Peterson

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Presence of bacteria in modified atmosphere packed raw chicken meat and their effect on shelf life and sensory characteristics – A microbiological and sensory evaluation

Bakteriehalter i rå kyckling förpackat i modifierad atmosfär och dess påverkan på hållbarhetstid och sensoriska aspekter – En mikrobiologisk och sensorisk utvärdering

Max Peterson

| Supervisor: | Sofia Boqvist & Karin Söderqvist, Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health |
|-------------|---|
| Examiner: | Hans Jonsson, Swedish University of Agricultural Sciences, Department of Molecular Sciences |

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Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences Department of Molecular Sciences

Abstract

Food waste is a huge challenge and constitutes a massive hurdle for development of sustainable food systems. Around 30% of all food produced for human consumption globally is estimated to be discarded, which in many cases is due to the foods having reached best-before-date. Bacterial groups such as Total aerobic count (TAC), Lactic acid bacteria (LAB) and Enterobacteriaceae (EB) are important quality indicators or spoilage bacteria of many food items, for example fresh and perishable foods such as chicken or minced meat. The aim of this study is to evaluate if the shelf life of chicken breast fillet can be extended by analyzing bacterial levels and sensory characteristics of chicken. In the present study, chicken breast fillets were analysed at three different time points; EXP (day or expiration), EXP+2 (2 days past expiration date) and EXP+4 (4 days past expiration date) at two different storage temperatures (4°C and 8°C) to see changes in growth of different bacterial populations over time from established expiration date up to 4 days past expiration date. In addition, two sensory evaluations were performed to evaluate flavor, odor and texture at the three different time points. The results from the microbiological study were compared to the sensory evaluation results in order to investigate the potential to extend the shelf life of chicken without compromising sensory characteristics. The highest population of TAC (8.1 log CFU/g) was found in chicken breast fillets stored at 8°C and analysed 4 days after expiration date. Chicken breast fillets that had been stored at 4°C and analysed at expiration date had the lowest TAC levels (5.9 log CFU/g). However, the sensory evaluations showed that none of the chicken breast fillets tested was significantly different to the other. This means that chicken with prolonged shelf life (and with TAC \leq 8.1 log CFU/g) was consumable and had no significant effect on flavor, odor or texture of the cooked product compared to chicken consumed at expiration date. Further research needs to be conducted to extend the shelf life of raw chicken breast fillets, and to establish a scientific foundation for appliance and use of dynamic food labels to reach the long-term goal of reducing food waste.

Keywords: broiler, expiration date, food waste, total aerobic count, *Enterobacteriaceae*, lactic acid bacteria, dynamic food labels.

Sammanfattning

Globalt matsvinn utgör ett stort hinder för utvecklingen av hållbara livsmedelssystem. Ungefär 30% av all mat som produceras för humankonsumtion globalt slängs, vilket i många fall beror på att livsmedlen som slängs har nått sitt bäst-före-datum. Bakteriegrupper som totalt aerobt antal (TAC), viktiga mjölksyrabakterier och Enterobacteriaceae är kvalitetsindikatorer och förskämningsbakterier i många livsmedel som exempelvis färska och lättskämda livsmedel liksom kyckling och köttfärs. Syftet med denna studie är att utvärdera om det går att förlänga hållbarhetstiden på kycklingbröstfilé genom att analysera bakteriehalter och sensoriska faktorer hos kycklingen. I denna studie har kycklingbröstfiléer analyserats vid tre olika tidpunkter; EXP (sista förbrukningsdag), EXP+2 (två dagar efter sista förbrukningsdag) och EXP+4 (fyra dagar efter sista förbrukningsdag) vid två olika förvaringstemperaturer (4°C och 8°C) för att se förändringarna i bakteriell tillväxt över tid från satt sista förbrukningsdag upp till 4 dagar efter sista förbrukningsdag. Utöver det har två sensoriska tester genomförts för att utvärdera kycklingens smak, lukt och textur vid de olika tidpunkterna. Resultaten från den mikrobiologiska delen av studien jämfördes med de sensoriska utvärderingarna för att undersöka möjligheten att utöka hållbarhetstiden på kyckling utan att äventyra sensoriska kvalitetsaspekter. Den högsta halten av TAC (8.1 log CFU/g) uppmättes i kycklingbröstfiléer förvarade i 8°C analyserade 4 dagar efter sista förbrukningsdag. Kycklingbröstfiléer som förvarats i 4°C och analyserades vid sista förbrukningsdagen hade den lägsta uppmätta halten TAC (5.9 log CFU/g). Däremot visade de sensoriska testerna att ingen av kycklingbröstfiléerna som testades skilde sig signifikant från varandra. Det betyder att kycklingen med förlängd hållbarhetstid (och med en TAC-nivå på ≤8.1 log CFU/g) var ätbar och att bakteriehalten inte hade någon signifikant effekt på smak, lukt och textur på den tillagade produkten jämfört med kyckling som sensoriskt testades vid sista förbrukningsdagen. Vidare studier behövs genomföras för att kunna förlänga hållbarhetstiden på rå kycklingbröstfilé och för att etablera en vetenskaplig grund för att kunna tillämpa och använda dynamiska datummärkningar på livsmedel för att nå det långsiktiga målet att minska matsvinnet.

Nyckelord: broilerkyckling, sista förbrukningsdag, matsvinn, totalt aerobt antal, *Enterobacteriaceae*, mjölksyrabakterier, dynamiska livsmedelsetiketter.

Table of contents

| List o | f tables | 8 |
|--------|--|-----|
| List o | of figures | 9 |
| Abbro | eviations | .10 |
| 1. | Introduction | |
| 1.1 | Slaughter process and bacterial contamination | |
| 1.2 | Spoilage bacteria | .14 |
| 1.3 | Intelligent labelling – dynamic food labeling | .14 |
| 1.4 | Aim and objective | .15 |
| 2. | Materials & Methods | .16 |
| 2.1 | Sample size calculation for microbiological analyses | .16 |
| 2.2 | Preparations of chicken meat and storage | .16 |
| 2.3 | Microbiological analysis | .18 |
| 2.4 | Bacterial identification | |
| 2.5 | Sensory evaluation | |
| | 2.5.1 Taste test | |
| | 2.5.2 Triangle test | |
| 2.6 | Statistical analysis | .23 |
| 3. | Results | .24 |
| 3.1 | Microbiological analysis | .24 |
| | 3.1.1 Total aerobic count population and change over time | .24 |
| | 3.1.2 Enterobacteriaceae population and change over time | .26 |
| | 3.1.3 Lactic acid bacteria population and change over time | .27 |
| 3.2 | Bacterial identification | .29 |
| 3.3 | Sensory evaluation | .30 |
| | 3.3.1 Taste test | |
| | 3.3.2 Triangle test | .30 |
| 4. | Discussion | .31 |
| 4.1 | Scope and challenges | |
| 4.2 | Hypothesis and results | |
| 4.3 | Bacterial identification | |
| 4.4 | Sensory evaluation | .33 |

| 4.5 | EU regulations and front pack labeling | 35 |
|-------|--|----|
| 4.6 | Limitations | 37 |
| 4.7 | Further research | 37 |
| 5. | Conclusion | 39 |
| Refer | ences | 41 |
| Popu | lar science summary | 47 |
| Ackn | owledgements | 49 |

List of tables

| Table 1. Changes of growth of total aerobic count in chicken breast meat stored at 4°C | |
|---|----|
| and 8°C measures in log CFU g-12 | 25 |
| Table 2. Changes of growth of Enterobacteriaceae in chicken breast meat stored at 4°C | |
| and 8°C measures in log CFU g-12 | :6 |
| Table 3. Changes of growth of lactic acid bacteria in chicken breast meat stored at 4°C | |
| and 8°C measures in log CFU g-12 | 28 |

List of figures

| Figure 1. Bacterial contamination and growth during slaughter | 13 |
|---|----|
| Figure 2. Flowchart from delivery to analysis | 17 |
| Figure 3. MALDI-TOF analysis | 19 |
| Figure 4. Taste test set up | 20 |
| Figure 5. Flowchart - taste test | 21 |
| Figure 6. Flowchart - triangle test | 22 |
| Figure 7. Total aerobic count | 25 |
| Figure 8. Enterobacteriaceae | 27 |
| Figure 9. Lactic acid bacteria | 28 |
| Figure 10. Bacterial presence. | 29 |
| | |

Abbreviations

| CFU | Colony Forming Units |
|-----------|--|
| EB | Enterobacteriaceae |
| EXP | Expiration Date |
| GMP | Good Manufacturing Practice |
| LAB | Lactic Acid Bacteria |
| MALDI-TOF | Matrix-Assisted Laser Desorption/Ionization Time-Of- |
| | Flight |
| MAP | Modified Atmosphere Packaging |
| MS | Mass Spectrometry |
| TAC | Total Aerobic Count |

1. Introduction

About 30% of all food produced for human consumption globally is lost or wasted throughout the supply chain (FAO 2011; Corrado & Sala 2018). In order to meet the global sustainability goals of Agenda 2030, there is a need to increase food production in a sustainable way and to promote sustainable food consumption (Grote et al. 2021). At the same time, there is need to reduce food waste while ensuring food safety and quality (UN 2015a; UN 2015b; Grote et al. 2021).

Food waste is defined as food items that are aimed for consumption but for different reasons do not get consumed (Swedish Food Agency 2021a). Wasted food does not only cause financial losses along the supply chain but it also has negative effects on the climate, for example by depleting natural resources and by emissions caused by transportation (Corrado & Sala 2018; Swedish Environmental Protection Agency 2013). Households are the main source of food waste and constitutes 53% of the total food waste in the European Union (Stenmarck et al. 2016). More than half of the food (60%) discarded in European households has been reported to be edible when wasted. At retail, 83% of the discarded food has been reported to be edible (ibid.). Looking solely on meat products produced in Europe, more than 20% is wasted or lost in the supply chain from production site to consumer level (FAO 2011). Consequently, one of the largest reasons for food waste is consumer and retailer wastage of foods that are close or are upon best before date (Vågsholm et al. 2020).

According to a survey performed by the Swedish Food Agency, 80% of the respondents believed they would throw less edible food if there was a more dynamic food labeling rather than today's standard with a fixed best before date or expiration date (2021b). In that case, could dynamic food labels be a solution for reduced food waste? Today's best before date could be misleading since it does not reflect upon the quality benefits of e.g., cool temperatures (4°C or lower) during storage of fresh food items such as raw chicken or milk. A lower storage temperature for fresh foods increases the shelf life of these products since the low temperatures suppress bacterial growth and survival (Swedish Food Agency 2021c, Swedish Food Agency 2021d). The difference between expiration date and best-before date is first and foremost the food item is safe to eat before the labeled date and best-before date guarantees that the food item is of its best quality before that date but can

possibly be consumed after that date as well (Swedish Food Agency 2021e; Swedish Food Agency 2022b). The European Union's legislative framework states what kind of foods can be labeled with either best-before date or expiration date. Chicken is considered a perishable food, meaning that it could be presumed to be a health hazard if consumed more than 10 days after slaughter. The regulations state that perishable foods must be labeled with expiration date because of the health risk (Regulation of the European Parliament and of the Council 2002/178; Regulation of the European Parliament and of the Council 2011/1169). The reason why chicken is included in these regulations is not motivated.

One way to reduce food waste could be to use dynamic food labeling. Dynamic food labeling or intelligent packaging could be a tool for retailers and consumers to determine when the food item is spoiled. Dynamic food labeling could be especially helpful when fresh and raw foods are stored at temperatures above 4°C. This is helpful since the expiration dates used today is based on a recommended storage temperature of 4°C (ibid.), at least regarding chicken and raw meat. A dynamic food labeling could also inform about the actual expiration date of the food item, if stored at optimum conditions, since the labeling is based on the actual bacterial growth in the packaging (Innoscentia n.d.). A more accurate and reliable front-pack label such as dynamic food labels, could prevent food at good quality from being wasted and could simultaneously ensure food safety (Restuccia et al. 2010).

1.1 Slaughter process and bacterial contamination

During slaughter, the chicken carcasses will be exposed to bacterial contaminants, which could contaminate the meat of the chicken, especially during evisceration (removal of intestines). The live and healthy birds' muscle tissues are sterile preslaughter but during the slaughter process, the muscle tissue is exposed to bacterial contaminants. There are certain differences between the slaughter of livestock, such as cattle and pigs, and the slaughter of poultry. Firstly, the process during chicken slaughter is mainly mechanical, while the slaughter of livestock is manual (Rouger et al. 2017). Secondly, water is used in several processing steps in chicken slaughter, which might facilitate contamination of the chicken meat by bacteria from the chickens' intestinal tract or skin (ibid.). Since chickens are small, there might be difficulties in mechanically fixating the carcasses in the slaughter process, which might lead to difficulties keeping surface areas and carcasses free from bacterial contaminants during the process of evisceration. The air, the equipment and surfaces in the slaughterhouse might also be sources of bacteria that could be transmitted to the slaughtered chicken muscle tissues (ibid.), especially if steps of disinfection and cleaning pre-slaughter have been insufficient.

During slaughter, chicken carcasses are eviscerated, and the contamination level is increasing during this step (Hinton et al. 2004; Göksoy et al. 2004). After that,

the chicken carcasses are scalded in water (50-60°C) to assist later step of feather removal by dilating feather follicles (Rouger et al. 2017). Scalding also diminishes the bacterial count on the chicken skins. However, the dilated feather follicles could possibly be a risk factor for bacterial contamination. Cool water baths used for chilling carcasses after feather removal has a washing effect diminishing bacterial counts on the chickens' skins, but it can also act as a bacterial transferring medium between carcasses (Hinton et al. 2004; Göksoy et al. 2004). The now water-borne bacteria could thereafter enter the dilated feather follicles of other carcasses and the cold water contracts the scalded dilated follicles, which encapsulates bacteria in the skin. In summary, the evisceration exposes the carcasses for bacterial contaminants, which are rinsed off during cold baths. Although, the bacterial growth on the carcasses such as in the feather follicles (see graphic explanation in Figure 1) (ibid.).

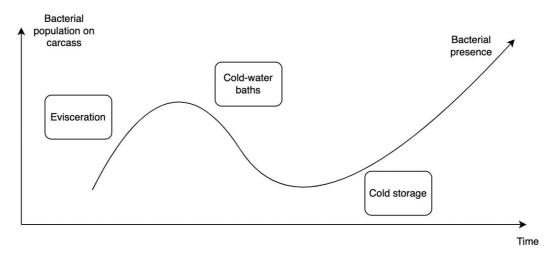


Figure 1. Bacterial contamination and growth during slaughter.

The figure above graphically shows how bacteria increases on the carcasses during the different steps of slaughter. The figure is principled and simplified thus does not represent actual levels of bacteria or steps performed at any slaughterhouse.

Chicken meat, depending on what cut you have, are stored in different packaging with different packing atmospheres. Chicken breast meat, which is the cut in focus in this study, is normally packaged in modified atmosphere packaging (MAP) with an anaerobic environment consisting of 30% CO₂ and 70% N₂ (Balamatsia et al. 2006, Marcinkowska-Lesiak et al 2015). A MAP solution with a low oxygen level will prolong the shelf life of many fresh meat products such as chicken and minced meat (Nordic Council of Ministers 2017; Economou et al. 2009; Swedish Food Agency 2022). The shelf life is prolonged since the growth of microorganisms thriving in oxygen-rich environments such as many spoilage bacteria, will be suppressed. The spoilage of the food therefore takes longer time. However, bacterial pathogens could still grow in this modified atmosphere but storage below

the recommended temperature for chicken, meat and fish products (below 4° C), will reduce the growth and activity of both pathogenic and spoilage bacteria, prolonging the shelf life (Swedish Food agency 2022a).

1.2 Spoilage bacteria

Some typical meat spoilage bacteria are lactic acid bacteria (LAB), *Enterobacteriaceae, Brochothrix thermosphacta* and *Pseudomonas* spp. (Rouger et al. 2017). In search for spoilage bacteria within the food production industry, total aerobic count (TAC) is commonly screened for (Chen et al. 2014). Total aerobic count includes all bacterial strains growing aerobically at a certain time and temperature and therefore microbiological analyses for TAC in foods often reveal a large variety of bacterial strains (Chen et al. 2014; Demaître et al. 2020; Bevilacqua et al. 2020). Screening for TAC and *Enterobacteriaceae*, can be used by food business operators to evaluate process hygiene and quality of their products. TAC level can also be used to evaluate the expected shelf life of a food item (ibid.), but it serves as a poor safety indicator since a food item with a low TAC count will not necessarily be free of bacterial pathogens such as *Salmonella* spp. and *Campylobacter* spp. in for example chicken meat (ibid.).

One of the reasons why food is wasted in households is poor knowledge of how to store different food items (Swedish Food Agency 2022b). Storage temperature plays an important role in the growth of spoilage bacteria. In a survey performed by Sveriges Radio (2011), only 40% of the respondents that answered that they knew what the temperature of their refrigerator was, kept a refrigerator temperature below the recommended 4-5°C. The higher temperature in the refrigerator, the higher risk for growth of spoilage bacteria (Casanova et al. 2022; Swedish Food Agency 2021c; Swedish Food Agency 2021d), which shorten the shelf life of food items such as meats (ibid.; Swedish Food Agency 2022b; Modin & Lindblad 2011). A MAP solution could, together with an optimal storage temperature, suppress the growth of spoilage bacteria since the high amount of CO_2 will inhibit the bacteria's ability to grow (Modin & Lindblad 2011).

1.3 Intelligent labelling – dynamic food labeling

Intelligent packaging is based on interactions between the packaging itself and the food item or its environment in the packaging (Restuccia et al. 2010). The purpose of that kind of technology is to inhibit microbial growth, delay oxidation and control respiration rate, and control moisture migration (ibid.). Other types of interaction technologies could be CO₂-absorbers or emitters, which could be included in dynamic food labels. The intelligent packaging solutions include, for example,

time-temperature indicators and gas indicators or gas sensors that could indicate the food item's changing quality (Ghaani et al 2016; Restuccia et al. 2010). The new techniques included in intelligent packaging can reduce the amount of food waste in many supply chains (Restuccia et al. 2010). Intelligent packaging can be adjusted to different types of food items and packaging solutions, which can guide producers, retailers and consumers to when these foods are edible (ibid.).

There are various types of dynamic food labels, and one type is based on reactive ink that is activated by gases produced the bacteria growing on the meat products within the packaging (Innoscentia n.d.). The more bacteria there is and the more they grow in number, the more gas they exude, and this will eventually activate the label. At a certain level of spoilage bacteria giving a certain concentration of exuded gas, the food item is deemed unfit for human consumption. The reactive ink of the dynamic food label would then change color to inform the consumer or retailer of the food items true expiration date (ibid.).

1.4 Aim and objective

One aim of this study was to evaluate if the shelf life of chicken breast fillet can be extended by investigating levels of spoilage bacteria on chicken stored at 4°C and 8°C at expiration date and at 2 and 4 days after expiration date. Another aim was to determine if the extended storage time would have any effect on sensory aspects such as taste and smell as well as texture and to link potential off-odors or off-flavors with levels of spoilage bacteria.

The specific research question in the present study was: (i) how does spoilage bacteria grow on the chicken breast fillets during MAP conditions over time at different temperatures and (ii) is there a level of log CFU/g that could be linked to poor quality from sensory evaluation that could be an indicator of actual spoilage of the product.

The results from this study contribute to the long-term aim, which is to reduce food waste with the aid of dynamic food labeling in order to reach global sustainability goals regarding sustainable food production and consumption.

2. Materials & Methods

The project was divided into two parts, a microbiological analysis, and a sensory evaluation.

2.1 Sample size calculation for microbiological analyses

Prior to the microbiological analysis, a sample size calculation was done taking factors such as expected difference in bacterial populations, power of the study, time and logistics into account.

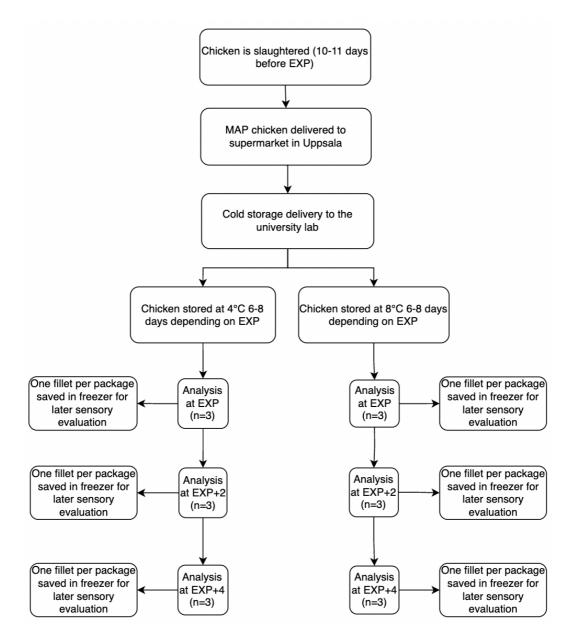
According to a previous study (Balamatsia et al. 2006), the standard deviation of log CFU/g for chicken samples was between 0.2 and 0.4. In this study, we wanted to detect a difference of 0.5 log CFU/g with an 80% probability (the power of the study). The sample size needed for each treatment (replicates of samples from the same temperature and storage time) was calculated to 11 samples assuming a standard deviation of 0.4. Since the plan for this study was to perform triplicates in five trials, this meant that the actual sample size was going to be 15 per treatment and therefore enough to assure that the statistical analyses would generate significant results.

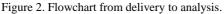
2.2 Preparations of chicken meat and storage

Chicken breast fillets packaged in modified atmosphere packaging (MAP) (30% CO_2 , 70% N_2) (Balamatsia et al. 2006) from one of Sweden's market-leader on chicken products were obtained from a local supermarket in Uppsala, Sweden. Each package contained two fillets and each fillet weighed around 150-200g. At each trial (there were five trials in total), 18 packages originating from the same batch¹ were transported chilled to the food safety laboratory at the Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences (Uppsala) the same day as it was delivered to the supermarket from the slaughterhouse, six to eight days before expiration date. Half of the

¹ One batch contains chicken from the same producer and has the same date of expiration

packages from each batch (n=9) were placed in an incubator set at 4°C and the other half at 8°C until time for analysis. Samples were analyzed in batches of six packages, three from 4°C storage and three from 8°C storage, and were tested on 0 (EXP), 2 (EXP+2) and 4 (EXP+4) days of storage post expiration date (see course of events in Figure 2). From each package of fillets, one was used in the microbiological analysis, and one was vacuum packed and kept frozen at -20°C for later use in the sensory evaluation study.





This flowchart covers the course of events from slaughter to delivery and analysis in the present study investigating bacterial presence and growth in chicken breast fillets over time. This scheme counts for all trials.

2.3 Microbiological analysis

From each chicken fillet, a sample of 25g targeting the surface of the chicken, was collected aseptically with scissor and tweezer and transferred to a stomacher bag. Further, 225ml Peptone water solution tempered to 25°C, was added and the bag was placed in a stomacher for homogenization of the sample for 120 s at room temperature. An eight step dilution series was performed using dilucups (Dilucups® Elegance; LabRobot, Sweden) and Petrifilms (3M Petrifilm[™], St Paul (MN), USA). Appropriate dilutions were applied to Petrifilms for samples from each temperature and time. *Enterobacteriaceae* was incubated at 37°C for 24±2h, while LAB and TAC Petrifilms were incubated at 30°C for 48±4h. After incubation, colonies on all Petrifilms were enumerated according to the Interpretation Guide (3M 2017). All plates were examined visually. Bacterial counts are presented as logarithmic values based on the CFU counts of each plate examined.

2.4 Bacterial identification

During the fifth trial of the microbiological part of the study, the TAC Petrifilm representing the highest dilution was selected for each sample from the different temperatures and time points (4°C and 8°C, EXP, EXP+2 and EXP+4). Colony material from five colonies was collected from each of these Petrifilms and spread separately onto one bovine blood agar plate. Blood agar plates were incubated at 30°C for 48h. After incubation, the agar plates were stored in a refrigerator (4°C) up to 5 days in order to maintain live bacteria on the agar until further analysis. Since there were mixed bacterial flora from colony material streaked onto agar in six occasions, the different colonies were tested separately. Upon analysis, colonies from the blood agar plates were collected with toothpicks and applied in duplicates onto metal 96-well-plates, costumed for MALDI-TOF-MS. One microliter HCCA matrix (α -Cyano-4-hydroxycinnamic acid dissolved in Bruker© standard solvent containing acetonitrile, water and trifluoroacetic acid) was added to each well. The wells were let to dry out before putting the 96-well-plate in the MALDI-apparatus, (see steps in Figure 3)

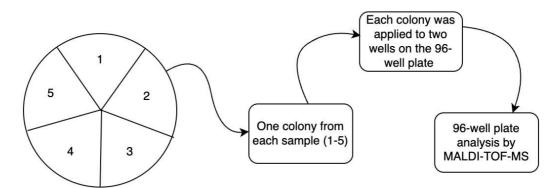


Figure 3. MALDI-TOF analysis.

Steps from pure-cultured isolate colonies on bovine agar plate to analysis by MALDI-TOF-MS. The 1-5 marks shows that in each separate part of the circle, there is a pure-cultured isolate that was collected from Petrifilms. From each pure-cultured isolate, one bacterial colony was collected and analysed by the MALDI-TOF-MS.

2.5 Sensory evaluation

2.5.1 Taste test

For the taste test, a coupled preference test with three pairwise comparisons for each test individual were used. The coupled preference tests were performed with 45 test individuals, including staff and students at lunchtime on one occasion in the canteen on the faculty of Veterinary Medicine and Animal Science at the Swedish University of Agriculture. Frozen chicken breast fillets from each time point (EXP, EXP+2 and EXP+4) at 4° C (see Figure 2), were thawed in their vacuum bags at 8° C over night. Prior to this test, a pilot study was performed to investigate how long the chicken needed to cook in sous-vide to reach an inner temperature of 72°C. The goal was to cook it as quick as possible without the chicken being undercooked at the same time as a good eating experience was desirable and avoid serving dry chicken to the test individuals. Results from the pilot study showed that 72°C for 1h was sufficient, and this cooking procedure was therefore used in the study. Every cooked fillet was sliced thinly into 20 pieces of around 10g each. Each test individual received six pieces of cooked chicken in six petri dishes (two from each time point; Figure 4). Three comparisons were performed: EXP versus EXP+2, EXP versus EXP+4 and EXP+2 versus EXP+4. The test individuals were asked to evaluate two samples at a time and register which samples they preferred logging into the EyeQuestion® (Amsterdam, the Netherlands) form. The order of the codes was randomized by EyeQuestion® and it was also stated which sample the test individuals should try first. In total there were 90 samples of each time point (EXP, EXP+2 and EXP+4). The samples from each time point were divided into two subgroups of 45 samples each and each received a three-digit code randomized by the sensory software program EyeQuestion[®].

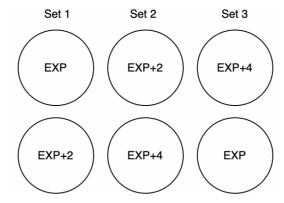


Figure 4. Taste test set up.

The taste test set up with six samples plates per individual. When performed, these plates were encoded. This order of samples/codes is an example since the order was randomized for each person participating in the testing.

Figure 5 describes the flow chart and the various steps included in the taste test.

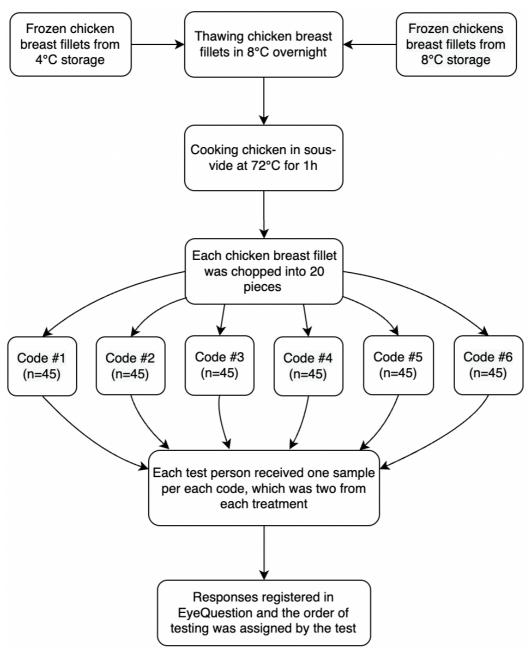


Figure 5. Flowchart - taste test.

This is a flowchart over the course of events of the taste test as one part of the sensory evaluation. The frozen chicken breast fillets were thawed at 8°C in a refrigerator one day before cooking in the sous-vide. The chicken was cooked in their separate vacuum bags.

2.5.2 Triangle test

This part of the sensory evaluation was a discrimination triangle test that was performed at the Department of Food and Meal Science at Kristianstad University. It was based on a triangle test design meaning every panelist received trials of three samples at a time. In EyeQuestion®, the panelists were obliged to choose one sample from each triangle, which they believed differed in flavor, odor and/or texture from the other two samples.

Eight trained panelists were presented samples of chicken meat from each temperature and time combination. The test was performed in duplicates, thus all panelist tested 15 triangles with 45 samples twice (divided into two sessions). The chicken was cooked sous-vide at 72°C for 1h and sliced into 20 pieces per filet (10-15g each). Each sample was given a random number by EyeQuestion®. The flow chart in Figure 6 describes the different steps in the triangle test of the sensory evaluation.

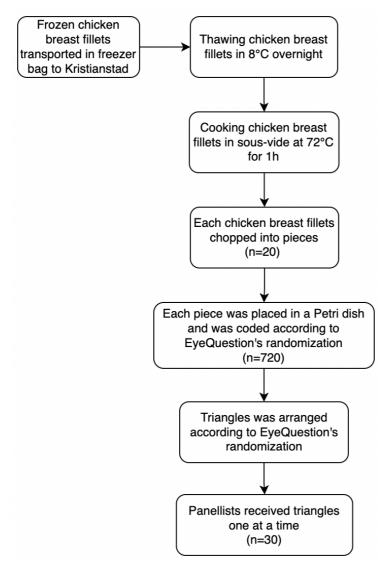


Figure 6. Flowchart - triangle test.

This flowchart displays the course of events during the triangle test performed at Kristianstad University. The first step is the transportation from Uppsala to Kristianstad by train where the chicken was kept in a freezer bag to minimize thawing on the way there. The thawing step on site in Kristianstad was done in a refrigerated room.

2.6 Statistical analysis

Results from the microbiological analysis was reported as mean values of the triplicates within each sampling time and the difference between the means were analyzed by ANOVA in RStudio® with a significance level of 95%.

QQ-plots for each bacterium (TAC, EB and LAB) were used to compare shapes of distribution of pairwise datasets using RStudio[®]. The plot indicated that the data was normally distributed.

Results of the taste test on campus Ultuna were also analyzed in EyeQuestionR[®] but with three pairwise comparisons tested once for each test person. Moreover, the results of the triangle test were analyzed by ANOVA in EyeQuestionR[®]. A significance level of 95% was used for both tests.

3. Results

3.1 Microbiological analysis

This part of the study focused on changes of bacterial populations of TAC, EB and LAB in fresh chicken breast fillet kept in MAP at 4°C and 8°C at three time points. In total, 86 packages, each containing two fillets, were included in the study. In total 86 chicken fillets were sampled for microbiological analysis and 86 were saved for later use in the sensory evaluation. Four packages were excluded; two were delivered with wrong expiration date and two developed a rapid growth of undefined bacterial colonies, which were visible on the chicken fillets. The means of the log CFU/g triplicates calculated for each sampling time and temperature is shown in Table 1 - 3.

3.1.1 Total aerobic count population and change over time

The average levels of log CFU/g for TAC, for each trial and combination of time and temperature are shown in Table 1. At EXP the level of log CFU/g of TAC, from all trials, was ranging between 5.90 and 7.68 at 4°C. At the same time point (EXP) at 8°C storage, the log CFU/g ranged between 7.38 and 7.85. After two and eventually four days, the log CFU/g increased in almost all cases. The highest log CFU/g value of TAC across all trials was ranging between 7.06 and 7.53 at 4°C of storage measured at EXP+4. At 8°C of storage, the log CFU/g ranged between 7.64 and 8.10 measured at EXP+4.

| Total aerobic count | EXP | | EXI | EXP+2 | | P+4 |
|---------------------|------|------|------|-------|------|------|
| Temperature | 4°C | 8°C | 4°C | 8°C | 4°C | 8°C |
| Trial 1 | 5.90 | 7.85 | 6.32 | 7.84 | 7.06 | 8.10 |
| Trial 2 | 6.53 | 7.56 | 6.85 | 7.71 | 7.53 | 7.98 |
| Trial 3 | 6.45 | 7.38 | 6.61 | 8.18 | 6.92 | 7.64 |
| Trial 4 | 7.68 | 7.74 | 7.33 | 7.81 | 7.51 | 8.09 |
| Trial 5 | 6.05 | 7.59 | 6.97 | 7.75 | 6.78 | 7.86 |

Table 1. Changes of growth of total aerobic count in chicken breast meat stored at 4°C and 8°C measures in log CFU g-1.

Each value of log CFU/g is the mean of each triplicate performed in each trial. EXP = day of expiration, EXP+2 = two days post expiration and <math>EXP+4 = four days post expiration.

In Figure 7, the results of the changes of TAC over time is visualized in a boxplot showing the differences between times and temperatures. From the statistical analysis it was shown that there was a significant difference in mean log CFU/g between the two applied storage temperatures (p < 0.0001). Also, there was a significant difference in mean log CFU/g between the three time points (p = 0.0004).

Furthermore, there is a significant difference (p = 0.0009) between the log CFU/g measured at EXP compared to EXP+4 at 4°C storage. At 8°C storage, there was no significant difference between the log CFU/g at any of the time points.

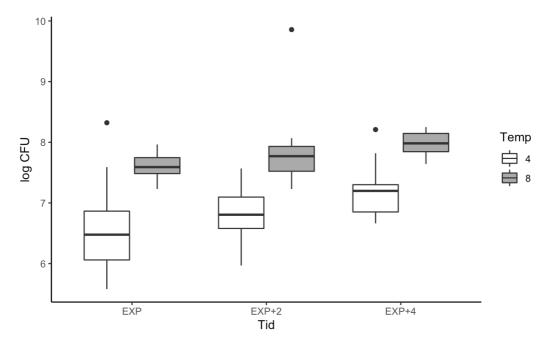


Figure 7. Total aerobic count.

In this graph is the change of log CFU/g in samples stored at 4°C plotted against the change in log CFU/g at 8°C storage. EXP = day of expiration, EXP+2 = two days post expiration and EXP+4 = four days post expiration. Dots are representing outliers of the microbiological results. Lines on each box represent the scatter of all log CFU/g measured. The line on the top represents the highest 25% of the measured log CFU/g values

for each combination of time and temperature. The bottom line of each box represents the lowest 25% of the measured log CFU/g values.

3.1.2 Enterobacteriaceae population and change over time

Table 2 shows that the level of log CFU/g value of EB on day of expiration (EXP) was ranging between 2.53 and 3.81 at 4°C of storage. At 8°C of storage of the same time point, the log CFU/g was ranging between 4.88 and 5.50. At last day of storage (EXP+4) the level of log CFU/g EB was ranging between 3.39 and 4.45 at 4°C of storage. At 8°C of storage at the same time point, the log CFU/g was ranging between 6.45 and 7.23.

Table 2. Changes of growth of Enterobacteriaceae in chicken breast meat stored at 4°C and 8°C measures in log CFU g-1.

| Enterobacteriaceae | EXP | | EXP+2 | | EXP+4 | |
|--------------------|------|------|-------|------|-------|------|
| Temperature | 4°C | 8°C | 4°C | 8°C | 4°C | 8°C |
| Trial 1 | 2.74 | 5.30 | 3.13 | 6.20 | 3.39 | 6.88 |
| Trial 2 | 3.09 | 4.88 | 3.54 | 6.54 | 4.45 | 7.23 |
| Trial 3 | 3.64 | 5.50 | 3.34 | 6.71 | 3.61 | 6.45 |
| Trial 4 | 3.81 | 5.19 | 3.47 | 5.89 | 3.78 | 6.61 |
| Trial 5 | 2.53 | 5.27 | 3.50 | 6.05 | 3.62 | 6.76 |

Each value of log CFU/g is the mean of each triplicate performed in each trial. EXP = day of expiration, EXP+2 = two days post expiration and <math>EXP+4 = four days post expiration.

Figure 8 shows that there was a significant difference in mean log CFU/g of EB between the two applied storage temperatures (p < 0.0001) as well as there was a significant difference in mean log CFU/g between the three time points (p < 0.0001). At the storage temperature of 4°C, there was a significant difference (p = 0.006) between the log CFU/g at EXP compared to EXP+4. There was no significant difference between EXP and EXP+2 nor EXP+2 and EXP+4 at 4°C. At the storage temperature of 8°C, there were significant differences (p < 0.0001) between the log CFU/g at EXP compared to EXP+4 at 4°C. At the storage temperature of 8°C, there were significant differences (p < 0.0001) between the log CFU/g at EXP compared to EXP+2 and EXP+4 at 4°C. At the storage temperature of 8°C, there were significant differences (p < 0.0001) between the log CFU/g at EXP compared to EXP+2 and EXP+4. There was also a significant difference (p = 0.0059) between the log CFU/g at EXP+2 and EXP+4.

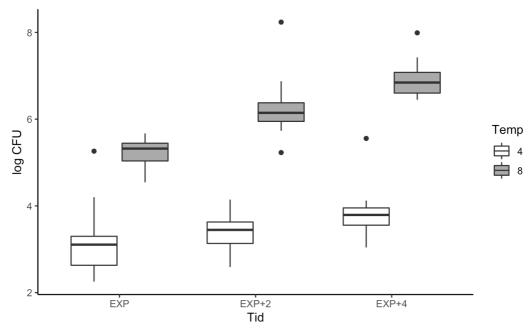


Figure 8. Enterobacteriaceae.

In this graph is the change of log CFU/g in samples stored at 4°C plotted against the change in log CFU/g at 8°C storage. EXP = day of expiration, EXP+2 = two days post expiration and EXP+4 = four days post expiration. Dots are representing outliers of the microbiological results. Lines on each box represent the scatter of all log CFU/g measured. The line on the top represents the highest 25% of the measured log CFU/g values for each combination of time and temperature. The bottom line of each box represents the lowest 25% of the measured log CFU/g values.

3.1.3 Lactic acid bacteria population and change over time

The level of log CFU/g LAB at EXP is shown in Table 3. The log CFU/g was ranging between 5.10 and 6.84 at 4°C of storage. At 8°C of storage at the same time point, the log CFU/g is ranging between 6.87 and 7.24. At last day of sampling (EXP+4), the level of log CFU/g LAB was ranging between 6.45 and 7.37 at 4°C of storage. At 8°C of storage at the same time point, the log CFU/g was ranging between 7.12 and 7.81.

| Lactic acid bacteria | EXP | | EXP+2 | | EXP+4 | |
|----------------------|------|------|-------|------|-------|------|
| Temperature | 4°C | 8°C | 4°C | 8°C | 4°C | 8°C |
| Trial 1 | 5.10 | 6.87 | 5.77 | 7.19 | 6.45 | 7.12 |
| Trial 2 | 5.94 | 7.21 | 6.19 | 7.04 | 7.37 | 7.81 |
| Trial 3 | 6.34 | 7.08 | 6.72 | 7.65 | 6.69 | 7.78 |
| Trial 4 | 6.84 | 7.24 | 6.64 | 7.59 | 7.37 | 7.73 |
| Trial 5 | 5.57 | 6.96 | 6.49 | 7.11 | 6.58 | 7.37 |

Table 3. Changes of growth of lactic acid bacteria in chicken breast meat stored at 4°C and 8°C measures in log CFU g-1.

Each value of log CFU/g is the mean of each triplicate performed in each trial. EXP = day of expiration, EXP+2 = two days post expiration and <math>EXP+4 = four days post expiration.

When analyzing LAB (see Figure 9) there were significant differences in mean log CFU/g between the two applied storage temperatures (p < 0.0001) and between the three time points (p < 0.0001). At 4°C, there was significant differences between EXP and EXP+4, and EXP+2 and EXP+4 (both (p < 0.0001)), and between EXP and EXP+2 (p < 0.01). At storage temperature of 8°C, there was a significant difference (p = 0.0003) between EXP and EXP+4.

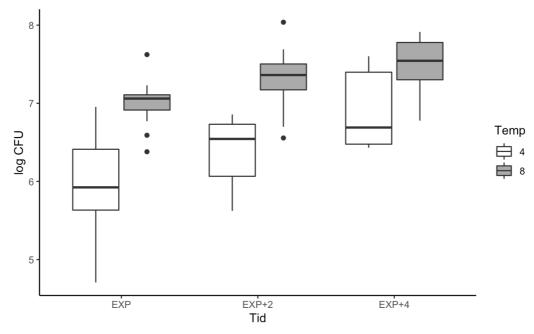


Figure 9. Lactic acid bacteria.

In this graph is the change of log CFU/g in samples stored at 4°C plotted against the change in log CFU/g at 8°C storage. EXP = day of expiration, EXP+2 = two days post expiration and EXP+4 = four days post expiration. Dots are representing outliers of the microbiological results. Lines on each box represent the scatter of all log CFU/g measured. The line on the top represents the highest 25% of the measured log CFU/g values for each combination of time and temperature. The bottom line of each box represents the lowest 25% of the measured log CFU/g values.

3.2 Bacterial identification

In total, the MALDI-TOF mass spectrometry analysis identified twelve bacterial isolates. As seen in Figure 10, Carnobacterium maltaromaticum and C. divergens was overrepresented in the analysis meaning they occurred in most samples tested. *Carnobacterium* spp. includes strains of lactobacilli that is commonly present in foods during fermentation (Cailliez-Grimal et al. 2014; Lorenzo et al. 2018) hence the occurrence in spoiling food. Other species identified were Brochothrix thermosphacta, Shewanella baltica, Serratia proteamaculans, Yersinia ruckeri, Hafnia alvei, Moraxella osloensis, Micrococcus luteus, Staphylococcus hominis as well as S. epidermidis. There was no clear pattern in occurrence of certain bacterial species linked to a certain time or temperature rather than that both C. maltaromaticum and C. divergens was present in most samples. However, bacterial species such as S. proteamaculans, Y. ruckeri and H. alvei was only to be found in samples stored at 8°C. Isolates from the chicken breast fillets that previously was described as spoiled due to visible colony growth on the meat surface were also analyzed. The bacteria on this chicken sample's surface presented to be a mix of C. divergens, Micrococcus spp., Pseudomonas fragi and Pseudomonas lundensis.

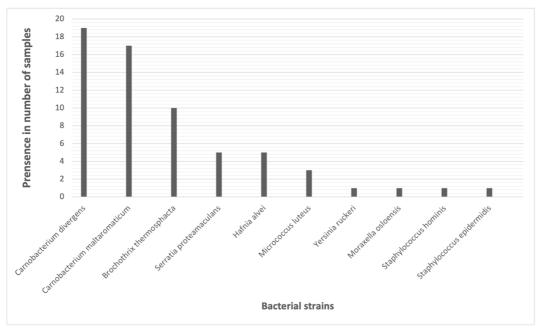


Figure 10. Bacterial presence.

The graph shows the number of samples each bacterial strain occurred in.

3.3 Sensory evaluation

3.3.1 Taste test

The taste test aimed to investigate if there is any specific time point that could be correlated with off-flavor and off-odors in the chicken samples. When run through EyeQuestion® there was no significant difference to be found between any of the pairs.

3.3.2 Triangle test

In this study, every treatment was compared with all other treatments as follow; EXP versus EXP+2, EXP versus EXP+4, EXP+2 versus EXP+4 for both temperatures. There was a significant difference (p < 0.05) found between the chicken samples from EXP+2 at 4°C storage and EXP+4 at 4°C storage, meaning that there was a significant difference in smell, taste and/or texture between these samples. No other comparison showed to have a significant difference between the two samples regarding sensory aspects in the evaluation.

4. Discussion

4.1 Scope and challenges

The aim of this study was to investigate the effect of increasing populations of spoilage bacteria over time, on chicken meat quality and sensory characteristics in order to link certain populations of bacteria to poor or good quality from a consumer perspective. The long term aim of this study was to reduce food waste by contributing to the development of dynamic food labels. A dynamic food label will inform the consumer and retailer about the real quality of the food item. It is based on bacterial populations within the packaging and will thereby replace the expiration date.

To make the present study feasible to conduct, given the time frame of this work, there was a focus on TAC, EB and LAB. TAC and LAB were chosen since they often occur on raw chicken or other meat products and affect the meat's quality, such as flavor and odor (Rouger et al. 2017). Total aerobic count is also a common quality indicator (Chen et al. 2014). Thus, this study contributes to improved knowledge about such bacterial quality indicators. However, since TAC only detects aerobic microorganisms, the actual total population of bacteria could be higher than what was shown in the present study. Furthermore, TAC analysis gives no indication on what types of bacteria are present in a sample, for example if they are pathogenic or not, which can make unsafe products pass through quality assurance steps in the production and process chains.

4.2 Hypothesis and results

The hypothesis of the present study was that chicken meat would be acceptable to eat, regarding flavor and odor of the cooked chicken breast fillets, also after the expiration date. According to previous studies on the matter, chicken and meat spoilage occur when TAC levels are around 7 log CFU/g sample (Höll et al. 2016; Rouger et al. 2017; Zhang et al. 2012). Although, this upper limit has not been fully supported by studies that have combined microbiological and sensory evaluation, suggesting this upper limit might or might not be accurate (Höll et al. 2016; Borch

et al. 1996). In this study the populations of TAC were close to 7 log CFU/g already on EXP (date of expiration) when stored at 4°C and between 7 and 8 log CFU/g when stored at 8°C, which suggests an acceptable level of TAC in the samples stored at 4°C but not at 8°C. The fact that the bacterial populations of TAC, EB and LAB at EXP were higher at 8°C than at 4°C reflect that the chicken breast fillet packages had been stored at these temperatures for 6-8 days before analysis. Previous studies have also shown that bacterial populations grow faster at temperatures over 4°C compared to temperatures below 4°C (Rouger et al. 2017; Zhang et al. 2012; Casanova et al. 2022).

As previously mentioned, a reason for a slower growth at 4°C compared to 8°C is the suppression of bacterial growth at lower temperatures. According to previous studies (Swedish Food Agency 2021bc; Swedish Food Agency 2021d; Swedish Food Agency 2022b; Casanova et al. 2022; Modin & Lindblad 2011) bacterial populations are suppressed and, in some cases, stopped when a food item is stored at cold temperatures. What is considered cold storage could be quite subjective though, since there are bacteria that could grow on chicken meat even in very cold storage. For example, *Pseudomonas* spp. and *Listeria monocytogenes* can grow in refrigerated storage temperatures (Fonseca et al. 2011; Meng et al. 2017).

The growth curves of EB were not following that of TAC. In contrary to the TAC, the growth curve for EB at 4°C was flatter than that of 8°C. This could possibly be explained by the fact that bacteria generally grow faster at warmer temperatures. Since many species of EB grows optimally at 37°C, temperatures higher than 4°C (such as 8°C) will logically lead to a higher growth (Borman et al. 1944).

Further on, looking into the microbiological analysis of LAB, there was relatively high numbers of this group of bacteria in all trials in the study. There were especially high numbers of LAB at 8°C at all time points. This could be caused by the fact that the bacteria have had long time to grow in a warmer temperature before analysis. This could lead to that the growth curve was approaching its peak-growth-point around the time of analysis, giving the flatter curve for the storage temperature of 8°C.

4.3 Bacterial identification

In the samples analyzed by MALDI-TOF-MS, *Carnobacterium divergens* and *C. maltaromaticum* were the most commonly identified bacteria, followed by *Brochothrix thermosphacta*. Since the colony material originated from the highest available sample dilution, the results reflect the most predominant bacteria in these samples. *Carnobacterium* spp. are lactic acid bacteria (Lorenzo et al. 2018) and *B. thermosphacta* belongs to the family *Listeriaceae*, however it is closely related to and was previously classified as LAB (Holley 2014; Feiner 2006). These bacteria

have been frequently isolated from meat and chicken (Höll et al. 2016; Rouger et al. 2017). They are recognized as common spoilage microorganisms in meat (ibid.; Lorenzo et al. 2018) and they can grow in raw meat products in all types of packaging e.g., vacuum and MAP (Höll et al. 2016; Casaburi et al. 2011; Doulgeraki et al. 2012). Regarding the occurrence of these spoilage bacteria in raw meat products included in the present study, it is likely that *C. divergens*, *C. maltaromaticum* and *B. thermosphacta* reflect the high general level of LAB, even though not all samples from all trials were analyzed. This assumption is based on previous studies showing that these bacteria are highly occurring in raw meat products (Höll et al. 2016). Furthermore, other bacterial species as *Hafnia alvei*, *Serratia proteamaculans* as well as *Yersinia ruckeri* were present in the chicken samples. These bacterial species are also commonly occurring in meat and chicken products during fermentation and spoilage (Höll et al. 2016; Rouger et al. 2017).

Only a subset of all chicken samples that were tested microbiologically were analyzed with MALDI-TOF-MS. This was due to economic and time constraints within the framework of this master's project. In total 123 pure-cultured isolates from the last trial of chicken samples were tested, representing samples from both storage temperatures at EXP, EXP+2 and EXP+4 during one out of five trials. It is however likely that the results obtained reflect occurrence of bacterial species also in all trials included in this project.

4.4 Sensory evaluation

Prior to both sensory evaluation trials, the samples were coded and randomized to keep the trial as unbiased as possible. By using randomization, it was made sure that the categories of samples were not connected to any of the codes and that a safety barrier was established to avoid flaws in the testing procedure, such as overlapping effects. The test panel at Kristianstad University tested 30 triangles of samples. If these triangles were to be tested in the same order (e.g., 1, 2, 3) every time (30 times), this could result in overlapping. An overlapping effect could lead to a misleading statistical result where the first sample probably would be more detailed analyzed by the panelists compared to the third sample. The effect of overlapping was reduced by randomizing the order of the sample codes in the test and the order of the triangle tests.

Both the taste test performed in Uppsala and the triangle test performed in Kristianstad generally showed that there were no differences in flavor, odor or texture between the samples at the different time points. There was only one comparison in the triangle test in Kristianstad that showed a difference in sensory characteristics between time points (EXP+2 and EXP+4 at 4°C storage). A reason for this significance could be the fact that the chicken breast fillets tested in the different sensory tests and in the microbiological analysis was not from the same

package nor the same batches. The natural variance between carcasses and batches could have been a reason for this significant difference for this comparison. Another possible explanation is that one of the chicken breast fillets in this comparison could have been contaminated with some sorts of bacteria along the production chain, leading to an altered bacterial flora in that fillet, which could affect the sensory sensation of this fillet. However, a general conclusion in this study is that there was no difference in quality and sensory aspects between chicken samples tested on expiration day and up to four days past expiration date. But why did the consumer panel not feel any significant difference between the samples EXP+2 and EXP+4 from the storage temperature of 4°C as the test panel in Kristianstad did?

The difference found in the triangle test for the samples EXP+2 and EXP+4 suggests that there was a difference between these samples that was not reflected by the microbiological analysis. A reason for this could be that the samples tested at Kristianstad University contained a bacterial species that was not present in other samples, that could have altered the sensory characteristics. Another reason for this could be the fact that the samples that were presented to the test panel was not necessarily the same samples that were analyzed microbiologically or tested in the taste test in Uppsala, even if they had the same treatment. While performing the microbiological analysis, the assumption was made that both fillets in one package had the same bacterial populations and the same bacterial species. This assumption made it possible to use one fillet for microbiological analysis and save the other fillet for the sensory evaluations. This assumption might be questioned since the two fillets in one packaging might not even come from the same bird even if the batch and the producer and slaughter date is the same. Also, since there were only two fillets in each package, the fillet that frozen for sensory evaluation could not be used for both the taste test and the trained panel test. Therefore, frozen fillets from different batches were used in the different sensory tests. In conclusion, this crosswise analysis of batches and fillets from different packages getting the same treatment might influence bacterial population within the packaging, which could have expressed different sensory characteristics when cooked and taste tested.

In contrast to the triangle test in Kristianstad, the samples tested on the consumer panel in Uppsala came only from the treatment of 4°C storage. The aim with testing the chicken samples on an untrained consumer group in Uppsala was to investigate if the flavor of the chicken samples differed for untrained consumers and not only for a sensory-trained panel. Since a skilled group has experience of testing many samples at a time, this group can handle a large quantity of samples without affecting the result or the quality of the test. This might not be the case for an untrained consumer group as in this case. Samples from 8°C was therefore not included in the test since the number of samples for each test individual would be too large to handle at once and since the samples stored at 4°C was of higher interest for the study. The optimum storage temperature for raw meat and chicken products is 4°C or below. The dynamic food label based on reactive ink that this study is supposed to contribute to developing, will be based on that the chicken is stored at the recommended temperature, which is 4°C or below.

4.5 EU regulations and front pack labeling

Chicken and other poultry are classified as food items that are highly perishable, meaning that they can be presumed as health hazardous for humans, post expiry (Swedish Food Agency 2021e; Swedish Food Agency 2022b; Regulation of the European Parliament and of the Council 2002/178; Regulation of the European Parliament and of the Council 2011/1169). Because of this hazard, food items such as chicken, must be marked with "expiration date" and not "best before date", according to EU law (Regulation of the European Parliament and European of the Council 2011/1169). The expiration date ("Sista förbrukningsdag" in Swedish) is based on the date of slaughter and informs that the chicken should be consumed within 10 days. Although, this time frame is difficult to find any scientific explanation for. The best-before date on the other hand is more flexible in that way that the food is presumed to be of its best quality prior to the set date. The bestbefore date opens for consumption of the food item even after the set date, which is contrasting to the expiration date where the food item is assumed to be a health hazard past the set date. There are many factors, both at retail and at household levels, such as insecurities about food labels, storage condition and food safety, that affect shelf life of raw chicken meat. This could lead to that set expiration dates and best-before dates, regardless of the food item, will not present the real date of expiry of that certain food item since it could have been stored incorrectly or there could be contamination in the production line that has affected its quality. A dynamic food label could therefore be a safer way of labeling when it comes to expiration and food going bad since these labels will present the real quality of the food item for the consumer or retailer in real time.

Based on visits to different supermarkets, it seems that the knowledge gap leads to retailers clearing shelves from chicken already before expiration date, which will contribute to unnecessary food waste of perfectly consumable chicken meat. Most of the retail managers said that chicken was not for sale in their store on its expiration date. Raw chicken products were off the shelves two days before expiration date. The fact that retailers throw out perfectly fine chicken, demonstrates the impact of the knowledge gap in this area today. Also, this management is partly constituted by consumer's attitude and trust in the current front pack labeling.

The fact that any other label than expiration date label is unlawful to use on foods such as chicken, creates a huge hurdle for the development of dynamic food labels for easily spoiled foods in Sweden and in the European Union. Although, it is not obvious that these "high-risk foods" will be health hazards even if consumed post expiry. Even if these foods can carry health hazardous microorganisms, most of the bacteria present are harmless and cold-insusceptible spoilage bacteria. These bacteria mainly affect the quality of the food item because of fermentation, leading to off-odors and off-flavors that humans associate with rotten food or food gone bad. If there are health hazardous microorganisms on the food item, they have likely originated from contamination at slaughter or during cross-contamination at a later processing step. The presence of health hazardous bacteria will not be changed due to the food reaching the date of expiry. Many of these health hazardous bacteria that could cause illness has very small or no growth at all during cold storage conditions. An exception is Listeriaceae that grows in refrigerator temperatures. Furthermore, chicken as well as other high-risk foods are normally heated prior to consumption, which means the bacteria that could have survived on the chicken, both hazardous and harmless spoilage bacteria will die off (Swedish Food Agency 2021e). But why are some foods classified as high-risk foods if they will be safe to consume after cooking? That is a question that is hard to answer by looking into regulations. Maybe it is classified in this way due to tradition or past ideals how to slaughter, process and prepare raw chicken, in this case. Many are intimidated by handling raw chicken and are afraid of catching any bacteria and turn sick from e.g., Salmonella spp., which is not a big issue in Swedish agriculture and poultry farming due to an extensive control program run by the national Food Agency in Sweden in accordance with the regulation of the European Parliament and of the Council (2010/254; Swedish Food Agency 2019). Other bacteria commonly present in raw chicken meat is Campylobacter spp. This family of pathogenic bacteria is highly associated with illness in case of human consumption. But again, if there is Campylobacter spp. on the surface of the chicken, they will die off in matter of seconds after cooking temperatures over 71°C and will not constitute a health hazard in case of human consumption of fully cooked chicken. Although, the risk of cross-contamination could constitute a health hazard if the contamination occurs after cooking. If the chicken is not handled properly and raw chicken comes in contact with cooked chicken, the cooked chicken might be unfit for human consumption if it is not cooked again. Furthermore, Campylobacter spp. populations does barely grow in refrigerator temperatures and could instead decrease in number at these temperatures, which means the bacterial population will not increase closer to expiration date if stored properly (Bhaduri & Cottrell 2004).

For dynamic food labeling as an industry and to establish a market for the products such as gas indicator labels, there is need for change of these legislative frameworks on a European level. Based on the regulations set today, the gas indicators or other dynamic food labels cannot be applied to raw chicken products of any kind. Thus, there is need for further research in the area to change the current legislation regarding food labeling and easily spoiled foods.

4.6 Limitations

The batches of chicken breast fillets included in the study did not necessarily originate from the same producer. This is a limitation for the microbiological and sensory evaluations since there could be natural differences in bacterial levels between the batches and between the fillets within the batches. This could influence the significance level of the statistical analysis of the microbiological and sensory evaluations. If the study were to be performed again, the chicken breast fillets would preferably originate from the same batch to make sure to diminish natural variations between batches, producers and slaughter facility handling. Although, a strength of this study is the fact that different batches were analyzed, which could give a fairer judgement of bacterial levels in chicken breast fillets in general.

Furthermore, the sensory evaluation could be affected by the fact that samples with the same treatment comes from different batches. Different batches mean different producers, which also could mean different slaughter facilities. All these steps could affect the bacterial flora on the carcasses, contributing to altered sensory sensation regarding flavor, odor and texture of the chicken.

4.7 Further research

If more research was done in the area and more studies could prove that chicken breast fillets, in this case, could be consumed post expiration date, this would contribute to reducing food waste by developing new ways of labeling food.

There are several areas that relate to the present study that could be further explored. One area could be to add an odor analysis of the raw chicken breast fillets. Prior to microbiological analysis, when the packages of chicken breast fillets are opened, the fillets could be odor tested by a sensory panelist group.

Such as study could contribute with important information if consumers would be willing to cook chicken breast fillets regardless of their odor when opening the packages. It might be that consumers are likely to throw away chicken fillets even if it tastes good after cooking due to bad smell of the raw product.

Another area for future research is levels of bacteria or spoilage levels, where the dynamic food label (gas indicator) should be activated and change color to inform consumers and retailers about the food item's current spoilage. If a study were to investigate where the label should be activated and start showing darker color, this demands a study prior to that investigating point of spoilage. An approximate point of spoilage could be concluded with a microbiological analysis together with a detailed sensory analysis including a separate odor test.

Lastly, another area of research could be an investigation of how the consumer would react to dynamic food labeling and if such labeling would affect their consumption behavior. The study would investigate the current consumer food consumption behavior as well as the behavior when exposed to dynamic food labeling. If such study showed a positive attitude to dynamic food labeling it would contribute to the development and appliance of dynamic food labels. It could also soften legislations regarding food labeling and food safety in the European Union today, since it would demonstrate the consumers' interest and the public good of saving food from being wasted.

5. Conclusion

In order to apply dynamic food labels such as gas indicators and other emerging intelligent packaging solutions onto foods classified as easily spoiled, there are legislations to be revised and further research to be performed.

This present study can conclude that no significant effect on flavor or odor was experienced upon consumption of cooked chicken breast fillets up to four days post expiration date.

Since this study has not performed an odor evaluation, there is no clear point of spoilage to be concluded based on the present study's result. However, the results suggest that fully cooked chicken breast fillet could be consumed without an effect of sensory sensation at the highest level of TAC, EB and LAB measured in this study (8.1 log CFU/g TAC).

Although, there are hurdles to overcome in order to apply dynamic food labels on perishable foods such as chicken. Regarding dynamic food labels such as gas indicators, as the type that this study is meant to assist in developing, they will not be possible to apply on perishable foods such as chicken breast fillets with the legislations and scientific support of today.

Further research is needed to conclude a clear point of spoilage and to establish the usefulness of dynamic food labels in order to develop applicable labels for fresh and perishable foods such as chicken meat. From the basis of these further studies, decision-makers such as the European Union and the Council could be influenced in taking on new legislations enabling use of dynamic food labels also on perishable foods to take a stand in the work of reducing global food waste.

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Popular science summary

Food waste is a huge challenge to reach several of the UN's 17 global sustainable development goals. One third of all food consumers bring home from the supermarket, is estimated to be wasted at household level. The food waste must be reduced in order to contribute to sustainable food production and food consumption. However, there might be a trade-off between food waste and food safety.

The hypothesis of this project is that the shelf life of certain food products could be extended without compromising food quality and safety, thereby reducing food waste. Dynamic food labels are based on the food item's actual quality regarding bacterial levels indicating spoilage and not on traditionally fixed expiration dates. One example of a dynamic food labels are gas indicators, which reacts to volatile acids (gasses) exuded from the bacteria growing on the surface of the food item within the packaging. The labels inform the consumer or retailer by visual communication (coloring of the label) at what stage of quality the food item is.

The study monitored the bacterial growth of chicken breast fillets at three different time points (at expiration date, two days past expiration date and four days past expiration date) stored in two different temperatures (4°C and 8°C) and compared the results with sensory evaluations of the chicken. The lowest level of bacteria was found in the chicken breast fillets stored at 4°C on the date of expiration. The highest level of bacteria was found in the chicken breast fillets stored at 8°C four days past the expiration date.

The results of this study showed that up to four days after expiration date of raw chicken breast fillets, the growth of spoilage bacteria did not have a significant effect on the cooked chicken's sensory characteristics such as taste, smell or texture. It can therefore be suggested that chicken heated to 72°C can be consumed safely and with no impact on quality aspects at the highest level of bacteria measured in this study, even four days after the expiration date set on the packaging today.

There is a need for further research on dynamic food labels before they can be introduced on the market, for example, studies about potential points of spoilage combined with studies on odor-based sensory evaluations on raw chicken products. If a foul odor of food products makes consumers throwing them away it does not matter if the product is safe to eat after heating. Also, the legislation is a huge hurdle for the development and appliance of dynamic food labels on foods such as chicken today. According to EU regulations, easily spoiled foods cannot be labeled with anything else than expiration date due to the presumed risk of consumers eating health hazardous chicken past expiration date. This calls for more research on the subjects to change regulations and to fill the knowledge gap of how spoilage and spoilage of these foods works.

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