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Research Article

Investigation of Yeast and Mould Growth Rate in Chopped Lamb Meat Packaged Under Different Systems during Refrigerated Storage

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Abstract

The objective of the present study was to investigate the growth rate of yeast and mould in chopped lamb meat packaged in air, modified atmosphere packaging (MAP) of two different gas mixtures (CO_2/N_2), air packaging with essential oils: thyme oil 0.1% (v/w), air packaging with thyme oil 0.3% (v/w), air packaging with oregano oil 0.1% (v/w) and air packaging with oregano oil 0.3% (v/w) during meat storage under refrigeration for a period of 25 days. Results showed that the growth rate of yeast and mould was high in air packaging. The upper microbiological limit for yeast and mould was considered that of 5 log CFU/g. The most pronounced effects were recorded for the MAP packaging, followed by those of oregano and thyme. MAP alone or in combination with essential oils is proposed as an innovative packaging technology for the yeast and mould control in raw lamb meat.

Keywords: Yeast; Mould; Lamb Meat

Introduction

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The first yeast originated hundreds of millions of years ago, and 1,500 species are currently identified [1]. These are estimated to constitute 1% of all described fungal species [2]. On the other hand, moulds are fungus that grow in the form of multi-cellular filaments called hyphae [3].

Yeasts are able to grow in foods with a neutral or slightly acidic pH environment and in the presence of sugars, organic acids, and other easily metabolized carbon sources [4]. During their growth, yeasts metabolize some food components and produce metabolic end products. This causes the physical, chemical, and sensible properties of a food to change, and the food is spoiled [5]. The growth of yeasts within food products is often seen on their surfaces, as in cheeses or meats, or by the fermentation of sugars in beverages, such as juices, and semi-liquid products, such as syrups and jams [4]. The yeast of the genus *Zygosaccharomyces* had a long history as spoilage yeasts within the food industry. This is mainly because these species can grow in the presence of high sucrose, ethanol, acetic acid, sorbic acid, benzoic acid, and sulphur dioxide concentrations [6] representing some of the commonly used food preservation methods.

Moulds, on the other hand, tend to grow on the surface of objects in the shape of a visible 'mycelium' made up of many cells. Moulds have both positive and negative effects on the food industry. Specific types of moulds are beneficial for the food industry since these are involved in Blue, Brie, Camembert, and Gorgonzola cheeses. Other types of moulds can be quite toxic and may produce allergic reactions and respiratory problems, or produce poisonous substances called mycotoxins. *Aspergillus* mold, for instance, which is most often found on meat and poultry (as well as in environment), can cause an infection called Aspergillosis, which is actually a group of illnesses ranging from mild to severe lung infections, or even whole-body infections. One of the greatest concerns regarding mould in food is the mycotoxins that some varieties produce. One of the most researched mycotoxins is aflatoxin, a cancer-causing poison [7].

A great concern then for both the food industry and scientific community is to develop methodologies for the control of yeast and mould in foodstuffs.

Lamb meat is a favorable foodstuff. The primary lamb and mutton consuming countries (on a per capita basis) are New Zealand, Australia, Greece, Uruguay, and Ireland. Lamb predominates in the

cuisines of Greece, Turkey, and the Middle East, commonly marinated and roasted on a skewer (shish kebab) or cooked with local vegetables. A classic Middle Eastern dish is ''kibbe'', a mixture of ground lamb and cracked wheat [8]. It is a good source of protein (ca.19g/100g), macro- and micro-minerals (potassium, phosphorous, sodium, calcium, magnesium, iron, zinc, selenium, etc.) and vitamins (thiamin, riboflavin, niacin, pantothenic acid, B6, B12 and D), while provides the human body with energy in the range of 250 - 300 kcal/100g when roasted, depending on the cuts consumed (leg, shoulder arm chop, shoulder blade chop, rib rack, loin chop, foreshank, etc.) [9]. Some typical species of microorganisms identified in lamb meat has been previously reported to be: *Staphylococci, Corynebacterium, Streptococci, Micrococcus, Salmonella, Escherichia coli,* Yeast and Mould [10,11].

Based on the aforementioned, the objective of the present research hypothesis was to investigate the growth rate development of yeast and mould in chopped lamb meat packaged in different systems: i) air, ii) modified atmosphere packaging (MAP1: 60% CO_2 - 40% N_2, MAP_2: 80% CO_2 - 20% N_2), air packaging with thyme oil 0.1% (v/w), air packaging with thyme oil 0.3% (v/w), air packaging with oregano oil 0.1% (v/w) and air packaging with oregano oil 0.3% (v/w) during meat storage under refrigeration for a period of 25 days.

Material and Methods

Preparation of lamb meat samples, packaging material, essential oils, microbiological analysis and instrumentation.

Details on preparation of lamb meat samples, packaging materials, essential oils, microbiological analysis and instrumentation used are given in a previous work [12]. The composition of gas mixture (CO_2/N_2) inside the package was measured throughout storage using a PBI Dansensor (Dansensor A/S, Model CheckMate 9900, Denmark).

Preparation of Rose-Bengal Chloramphenicol agar

Approximately 28.5g of powder was weighted in a SIMAX vial (1000 mL, Czech Republic) and dispersed in 1 liter of deionized water. The mixture was left to soak for 10 minutes, swirled to mix well and was then sterilized for 15 minutes at 121°C in a lab-scaled autoclave. After sterilization, the mixture was left to cool at 47°C in a thermostated water bath (BioLine Scientific, Greece) and then 2 vials of X009 chloramphenicol were added. The final mixture was mixed well and was then poured into Petri dishes [13].

Enumeration of Yeast and Mould

Yeast and mould were determined by surface spreading of the decimal dilutions of lamb meat samples, on rose-bengal chloramphenicol agar (LAB 036. LAB M, Lancashire, UK) after incubation at 30°C for 3 - 5 days. Due to the negative geotropism that some moulds show, the plates were not incubated inverted, as in bacteria. White or semi-white and in some cases coloured colonies were measured. In all cases, a Gallenkamp (Ontario, Canada) colony counter was used to enumerate the micro-organisms and the counting was made only on those plates having 10 - 300 colonies. For each treatment, 3 Petri dishes were prepared. Results were expressed as log CFU/g.

Statistical analysis

Statistical analysis was performed using SPSS statistics software (Version 20 for Windows, Armonk, NY, IBM Corp.). Graphs were produced using Microsoft Excel 2007. Microbiological data were transformed into logarithms of the number of colony forming units (log CFU/g). Then, the average values of each treatment, with respect to storage time, were subjected to T-test. The level of significance was set at $p \leq 0.05$.

Results and Discussion

Usually, even in small populations, yeast and mould can create large-scale colonies, resulting in the organoleptic rejection of a product. Therefore, suspending these organisms in a modified atmosphere is important for the quality and safety of products. This observation is of particular importance given that several yeast and mould forms show heat-resistant and may survive during the heat treatment of the product.

Aerobic packaging is generally a good environment for the growth of yeasts and moulds. As shown in figure 1 after a relatively short incubation phase, these are multiplied rapidly and surpass the level of 5 logCFU/g at 9^{th} day for air packaging and the 13^{th} day for air packaging with 0.1% v/w thyme oil. The higher concentration of thyme oil used [0.3% (v/w)] showed a more efficient result ($p < 0.05$). Oregano oil used at both concentrations retained the growth rate of yeast and mould lower than 5 log CFU/g for a period of 13 - 14 days. The most significant effects ($p < 0.05$) were recorded for both MAP packaging, especially MAP2 (80% CO_2 - 20% N_2), in which the microbiological level of yeast and mould remained lower than 5 CFU/g. Given the toxicity that some species (especially moulds) may possess the upper limit was considered as the 5 log CFU/g.

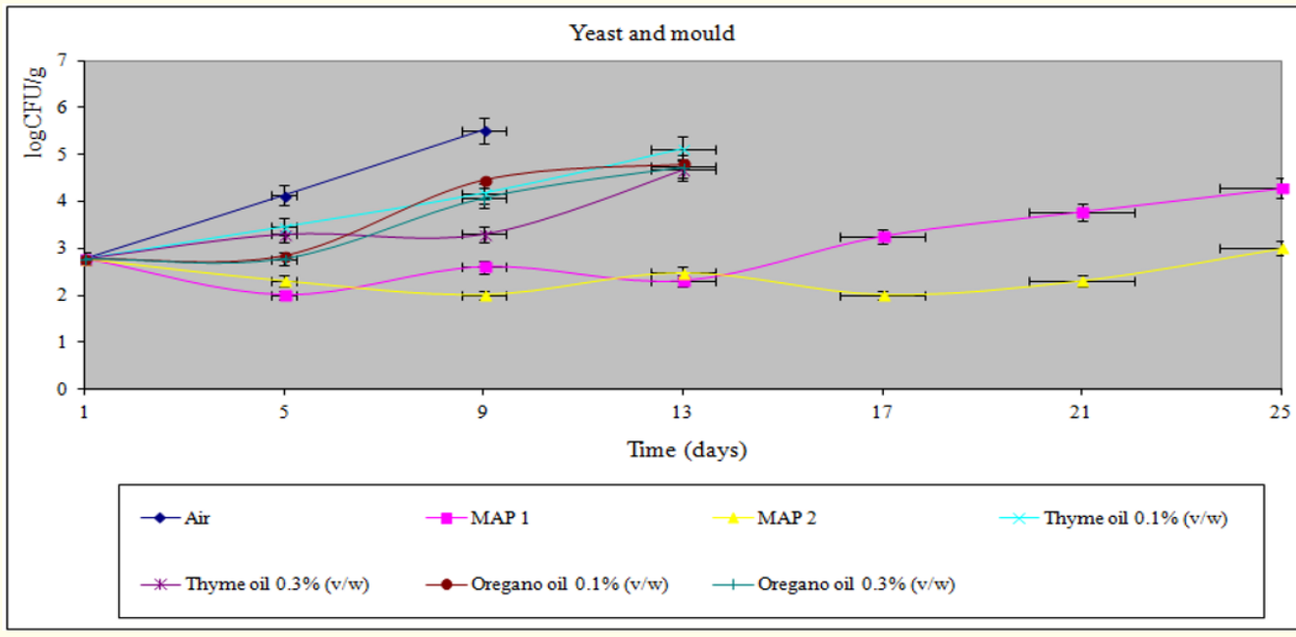

Figure 1: Growth rate of yeast and mould in chopped lamb meat packaged under different systems during refrigerated storage. Error bars are provided at the confidence level $p \leq 0.05$.

In addition, total viable counts in air packaging exceeded the upper microbiological limit on days 5 and 6 of refrigerated storage along with the organoleptic rejection of the product on day 9 [9], no measurements were carried out for yeast and mould after the 9th day for lamb meat samples packaged in air. Furthermore, the gas mixture composition was slightly altered during storage (CO_2 was decreased and N_2 was increased) showing that an effective atmosphere packaging was followed.

Before going any further, it is important to mention that the reduction in the volume of packaging, it is owed to the diffusion of CO_2 both in the aqueous and fat phase of lamb meat samples (CO_2 is both soluble in water and fat). The increase in the N_2 percentage composition could be characterized as a ''virtual reality'', because it is actually calculated by subtraction of 100% of the total. Nitrogen is used to replace O_2 in the modified atmosphere packaging and to delay oxidative rancidity. It is practically insoluble in both water and fat (very low solubility). The presence of O_2 was in traces (ca. 0.03%) during the whole storage.

In a previous work dealing with the extent of contamination of yeast and mould in chilled foodstuffs low numbers of colony forming units (2 - 4 log CFU/g) were recovered from bacon and some types of fresh sausages, whereas high numbers (4 - 6 log CFU/g) were observed for minced meats, burgers prepared from pork, lamb, beef and turkey burgers, fresh or processed poultry meat. The majority of meat samples had pH values of 6 - 6.5, whereas most fresh sausages and some burgers had an average pH value of 6.6 [10]. Such pH meat values are in accordance with a previous work on lamb meat [12].

Regarding the use of MAP, present results are in agreement with previous studies in which the modified atmosphere packaging (80% CO_2 - 20% N_2) inhibited the growth of yeast and mould [14], whereas the addition of thyme oil had a strong inhibitory effect on the growth of these microorganisms [15].

Conclusion

MAP served as the optimal medium for the control of yeast and mould growth rate in chopped lamb meat packaged in LDPE/PA/LDPE films, during refrigerated storage for a period of 25 days. Thyme oil 0.3% (v/w) was the most effective essential oil in decreasing the populations of yeast and mould among the other essential oils used for a period of 13 days. Future perspectives may include the combination of MAP with numerous essential oils of different concentrations (thyme, oregano, clove, wild lavender, throubi, etc.) in order to investigate whether a more efficient decrease on populations of yeast and mould grown in lamb meat, during refrigerated storage, may be achieved.

Conflict of Interest

The author needs funding to carry out the research product entitled: ''Control of the spoilage flora of lamb meat using innovative approaches and chemometrics''.

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