EFFECT OF MICROBIAL STARTER CULTURE ON SOME MICROBIAL PROPERTIES OF FERMENTED SAUSAGE

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ABSTRACT

The study was aimed to investigate the effect of microbial starter culture on some microbial properties of fermented sausage, the mixed starter culture which was used were composed from Lactobacillus curvatus, Pediococcus acidilactici and Staphylococcus xylosus after study microbiological analysis of sausages and counting total viable count, lactic acid bacteria (LAB) of sausages and sausages yeasts and molds counts (log CFU/g )at fermentation period (0,5,10) day and after stored period for (30,60,90) days for control and starter inoculated sausages were the obtained result the total viable count increased respectively from zero time to end day of production which was 5.44 to 8 (log CFU/g ) for control and 5.88 to 8.37 (log CFU/g ) for starter inoculated sausages after these period at the stored period the number decreased. The Lactic acid bacteria (LAB) counting done and the range increased respectively from zero time to 10th day of production was 4.67 to 8.13 (log CFU/g ) for control and 6.11 to 8.7 (log CFU/g ) for starter inoculated sausages after these period at the stored period the range of LAB decreased however the yeast and mould counting undertaken for both type of sausages and they was lowered from first day till end day of storage from zero time to end day of stored was 5.32 to 3.78 (log CFU/g ) for control and 5.28 to 3 (log CFU/g ) for starter inoculated sausages.

Keywords: starter culture, total viable count, lactic acid bacteria (LAB) of sausages, sausages yeasts and molds counts

INTRODUCTION

Fermented sausage is prepared from seasoned, crude meat that's stuffed in casings and is allowed to ferment and mature (1,2). Inoculation of the sausage is a batter with a starter culture composed of chosen lactic acid bacteria (LAB), i.e. homofermentative lactobacilli and/or pediococci, and Gram-positive, catalase-positive cocci (GCC), i.e. nonpathogeni, coagulase-negative staphylococci, improves the quality and safety of the final product and standardizes the production process (1-3). Various microorganisms at the environment and raw material are source of microorganisms; naturally contaminate dry sausage mixtures (4). Among them, lactic acid microorganisms (LAB) are responsible for the most event during dry sausage

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Ripening the fermentation of carbohydrates. Can lead to the improvement of normal flavours and tastes through by lactate oxidation, proteolysis, degradation of amino acids and lipolysis (5).

Fermentation is a worldwide and ancient preservation technique, possibly one of the oldest strategies recognized (6). It is generally employed to keep or enhance the organoleptic attributes and microbiological safety of foods. Indigenous microorganisms have been responsible for fermentation traditionally, however starter cultures can now be brought to set off fermentation and favorable processing conditions can be chosen to make sure desired best quality (1,7). These techniques encourage the improvement of a suitable safe microflora, which is vital for preventing the outgrowth of spoilage microorganism and food-borne pathogens (8). The major function of lactic acid bacteria is to convert fermentable sugars in the sausage batter to lactic acid, thereby contributing to product safety via developing unfavorable conditions for pathogens and spoilage organisms. (9, 10). However Staphylococcus and Kocuria (formerly Micrococcus) are the main representatives of this family. These micro-organisms have important enzymes like some proteases and lipases that contribute to flavor generation. (11). Hydrolysis, the partial degradation of the lipid fraction in fermented sausages, is one of the most important biochemical processes, which takes place as a result of the action of glycerol ester hydrolases (12-15).

MATERIALS AND METHODS

Sausage Preparation And Fermentation Procedure

The sausages were produced with a simple modification according to (16-18) lean beef (80% w/w) and animal internal tissue fat (20% w/w). Meat and animal internal tissue fat were minced and mixed with sausage spices obtained from local market added bay range (3.5 g/100 g), salt (2 g/100 g), raw garlic paste (0.2 g/100 g), and crystal sugar (0.3 g/100 g). Half of the obtained mixture was inoculated with Safe Pro F-LC starter culture which for each 25-gram packet of F-LC starter culture is sufficient for 100 kg of meat about 10 cfu/g; mixed culture, Safe Pro F-LC starter, Chr. Hansen, Germany starter cultures Lactobacillus curvatus, Pediococcus acidilactic and Staphylococcus xylosus in a convenient freeze-dried And another half use as a control without starter. The mixture stuffed in natural beef casings the ripening was performed as follows in a traditional method for 10 days (control: C and starter inoculated: S). The conditions natural conventional maturation room the primary arrange comprised of 2 days drying with the relative humidity (RH) of 85% and a temperature of 22°C. Airspeed velocity (m/s) 0.1-0.2 that was at that point diminished to 15-17°C were the temperature lowered after that 5 days to (normal 7.11 ± 3.20°C) and relative humidity average 75 to 80 %). And after 10 days fermentation Sausages were packed in vacuum bags and stored at refrigerator at 2-4°C up to 90 days.

Microbiological Analysis

Preparation Of Samples

Microbiological Investigation For the microbial analysis, 10 g of each test were blended with 90 mL of saline/peptone water (1 g/L peptone) and homogenized. Through mixing in stomacher 400 circulator for
1.5 min at 260 pm. Hence, decimal dilutions were arranged with 0.1% (wt/vol) peptone water and plated, in duplicate, on the corresponding media\(^{19}\).

**Enumeration Of Total Viable Count (TVC)**

Standard plate count was done as according to \(^{20}\). Plate count agar has been prepared and sterilized as per the manufacturing directions. In pre-sterilized plastic petri, one ml of each sample dilution prepared, in the item (3.2.6.1) were inoculated, The medium was poured at 45°C of around 12-15 ml for each plate over the sample dilution, and blended delicately until it was dispersed. The plates are incubated for 72±3 h at 30±1°C. Calculated the number and expressed as CFU/g sausage.

**Lactic Acid Bacteria (LAB) Count**

Lactic acid bacteria (LAB) count Man Rogosa Sharpe (MRS) Agar (OXOID, CM361) was used as medium. Double layer cast planting. Sample incubated at 30°C for 72 hours at anaerobic condition\(^{17}\).

**Enumeration Of Total Yeast And Molds**

Yeast and mold count was done as described in the international standardized NO,6611 according to \(^{28}\). DRBC agar as described by the manufacture was prepared, sterilized, and cooled to 45±1°C. The medium was once poured nearly 12-15 ml for every plate over the sample dilution, and blended gently till it was once dispersed. Two plates have been incubated at 25°C for 5 days.

**Statistical Analysis**

Statistical analyses for all sausage samples were performed using SPSS statistical software (Version 22, IBM), analysis factorial -Test was used to compare in between the control and fermented sausage by starter by two factorial design, and of storage periods were evaluated by applying ANOVA according to a factorial design with repeated measurements in time. All figures are shown by mean values with the standard deviation (means ± SD).

**RESULTS AND DISCUSSION**

**Microbiological Analysis of Sausages**

**Total Viable Count (TVC)**

Sausages total viable counting done and the number expressed as (log CFU/g). Results during the fermentation stages of sausages are shown in Figure 1. TVC numbers in the stored period is given in Figure 2. The TVC numbers of the samples and the changes in the fermentation step versus time statistically significant (p <0.01). Sample type x Production time also have a significant effect on TVC (p <0.01). TVC number of the samples before fermentation was 5.44-5.88 log CFU/g for control and sausages inoculated with starter cultures at first day. With fermentation TVC number of sausages increased and this increase was statistically significant it was found to be important (p <0.01). On the fifth day of fermentation TVC numbers ranged between 7.46-7.68 log CFU/g for control and sausages inoculated with starter cultures. At the study
on fermented sausages (21) mentioned a TVC was 5.5-6.2 log CFU/g initially number increased to over 8.0 log CFU/g for fermented sausage. (17) the Italian type fermented sausages TVC 5.0 log CFU/g in the first and then increased to reach 7.0-8.0 log CFU/g after 14 days of the production.

Highest bacteria number mentioned in last day of fermentation and was 8.8-3.7 log CFU/g for control and sausages inoculated with starter cultures shown at Fig.1. The results agree with (22) reported that the LAB number in the first day of production was 4.36 log CFU/g for control sausages against 7.37 log CFU/g for inoculated ones also The highest level was found on the day 14 of production and then a slight decrease was observed to reach at the end of ripening levels of 8.13 and 7.14 log CFU/g respectively for inoculated and for control sausages.

![Graph showing ripening period and total viable count (TVC) in log CFU/g](image)

Fig. 1: Effect of starter culture and ripening period on Sausages TVB count (log CFU/g)

TVC numbers decreased during stored period (Fig 2). Differences in TVC numbers between samples and stored time x sample type Interaction was also statistically significant (p <0.01). The decrease in bacterial counts of the samples was 7.9 for control sausages 8.11 sausages inoculated with starter cultures found to be statistically significant on the 30th day.(P <0.01). On the 60th day of stored, TVC counts of samples decreased to 6.5-6.87 log CFU/g for control and sausages inoculated with starter cultures. On the last day of stored samples have the lowest TVC (5.57-5.8 log CFU/g ) control and sausages inoculated with starter cultures respectively and at the all steps sausages inoculated with starter cultures highest log CFU/g in the control sample shown at Fig 1 and Fig 2. Resemble with the (23) TVC numbers decreased in the sausages stored period, and TVC at the end 60th day of stored of TVC numbers was 5.0 log CFU/g.
Lactic Acid Bacteria (LAB) Of Sausages

LAB number of the samples at first day fermentation was 6.11 log CFU/g for control 4.67 log CFU/g and sausages inoculated with starter cultures. At the fifth day of fermentation, sausages numbers of LAB increased and this increase was statistically significant (p <0.01). On the fifth day of fermentation, LAB numbers ranged between 7.9-7.53 log CFU/g for control and sausages inoculated with starter cultures. Sample type x Production time also have a significant effect on LAB (p <0.01). Results during the fermentation stages of sausages are shown in Figure 3. LAB numbers in the stored period is given in Figure 4.

Lactic acid bacteria in fermented meat products they ferment sugar to lactic acid (24-26), depending on the ripening temperature of the LAB number of sausages the second day of fermentation 3.5-4.3 log CFU/g, and 8.0 log CFU/g are reported at last day of fermentation the pH values the lowest level of the samples depending on the number of LAB during this period.

(21,27) reported On the 7th day of production, LAB number that they were above 8.0 log CFU/g. (23) that the number of sausages LAB between 5.5-7.0 log CFU/g variance between them was detected. Different results between the researches, the researchers’ different starter culture and different maturing conditions may be applied. Highest LAB number reached at in 10th day of fermentation was 8.13-8.7 log CFU/g for control and sausages inoculated with starter cultures.
Decrease in the number of LAB counts of samples type at stored x time Interaction was also statistically significant (p < 0.01). The decrease in LAB counts of the samples was 8.65 log CFU/g for control sausages 8.1 log CFU/g for sausages inoculated with starter cultures found to be statistically significant on the 30th day (P < 0.01). On the 60th day of stored LAB counts of samples decreased to 6.89 - 7.53 log CFU/g for control and sausages inoculated with starter cultures. On the last day of stored samples have the lowest LAB (5.1-5.4 log CFU/g) control and sausages inoculated with starter cultures respectively and at the all steps sausages inoculated with starter cultures highest log CFU/g in the control sample shown at Fig 3 and 4. The results near with (28) reported Initial counts of LAB during stored 30 days was 5.17 log CFU/g and decreased after 60 days stored to 4.30 log CFU/g.
This slight decrease of LAB during stored is probably due to the lowering of fermentable carbohydrates (29) and the decrease of water activity (30). LAB were the dominant microflora at the end of the ripening for both sausages. this result confirms the good adaptation of LAB to the sausage and their faster growth rates during production and ripening of sausages (31-38).

**Sausages Yeasts and Molds Counts**

Sausages yeasts and molds counts of the samples at first day fermentation was 5.32 log CFU/g for control 5.28 log CFU/g and sausages inoculated with starter cultures. At the fifth days fermentation sausages numbers of yeasts and molds counts decreased and this decrease was statistically significant it was found to be important (p <0.01). On the fifth day of fermentation LAB numbers ranged 4.9-4.1 log CFU/g for control and sausages inoculated with starter cultures. These values are lower than the values of (23, 27, 39) reported the average yeast number of sausages after drying is 6.72 log CFU/g.(23) depending on the stored temperature of the number of yeast-mold It was between 2.5-4.5 log CFU/g.
At the 10\textsuperscript{th} day this number increased to 4.6 log CFU/g for control and 4.0 log CFU/g for inoculated samples. Sample type x Production time also have a significant effect on yeasts and molds (p < 0.01). Sausages yeasts and molds counts at the fermentation stages and stored period of sausages are shown in Figure 7. In the stored period is given in Figure 8. The results agree with (22) reported that the number of yeasts and molds increased from about 3 log CFU/g to reach about 4 log CFU/g for both inoculated and control sausages and was not affected by the addition of starters (p > 0.05). This evolution compare well with most studies on fermented sausages (31,34,36,40). Different researcher Inadequate prepared sausage can become contaminated with microorganisms due to technological processes and the use of such spices deteriorates the quality of meat products was reported to be reduced (41).

The number of yeasts and molds counts of samples Decrease at stored from 30 to 90 days and lowering count of the type at stored x and time Interaction was also statistically significant (p < 0.01). The decrease in yeasts and molds counts of the samples was 4.11 log CFU/g for control sausages 3.5 log CFU/g for sausages inoculated with starter cultures found to be statistically significant on the 30th day (P < 0.01). On the 60th day of stored yeasts and molds counts of samples decreased to 3.9-3.22 log CFU/g for control and sausages inoculated with starter cultures. On the last day of stored samples have the lowest yeasts and molds (3.78-3 log CFU/g) control and sausages inoculated with starter cultures respectively. The results near with (Gök, 2006) reported Initial counts of yeasts and molds counts during stored 30 days was 3.35 log CFU/g and decreased after 60 days stored to 3.20 log CFU/g.
CONCLUSION

The study was designed to investigate the effect of microbial starter culture on some microbial properties of fermented sausage which obtained results show as the total viable count and lactic acid bacteria was increased at the production period and decreased at stored period for the both type of sausages but control counting was lower than starter inoculated sausages count and however yeast and mould was decreased at the production period to end stored period for the both type of sausages but control counting was higher than starter inoculated sausages count.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


41- Kayaardı, S. and Gök, V., Use of ionized radion rays in meat and meat products. YY.University Institute of Science and Technology Publications, 1999.4; pp34–53.