J.Meat Sci., 2012, 8(1&2) : 1 - 7 Research paper

A Study on the Quality of Pork Sausages during Fermentation with Different Starter Cultures

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ABSTRACT

In this study, proximate composition, physico-chemical and microbiological characteristics were evaluated during fermentation and drying of pork sausages using different Lactic acid bacteria viz., *Lactobacillus casei (LC), Lactobacillus plantarum* (LP) and *Pediococcus pentosaceus (PP).* The per cent moisture of different meat sausages inoculated with lactic acid bacteria decreased significantly (P>0.01) whereas, per cent protein and fat increased during fermentation and drying. Among treatments, significantly (P>0.01) lower moisture and higher protein and fat content were observed in sausages treated with LP than PP and LC. Physico - chemical characteristics, pH, water holding capacity (WHC), water activity and cooking yield of pork sausages inoculated with Lactic acid bacteria were significantly (P>0.01) lower whereas emulsion stability and shrinkage were significantly higher than control after fermentation and drying. Among treatments significantly (P>0.01) lower pH, WHC, water activity and cooking yield and higher emulsion stability and shrinkage were observed in sausages treated with LP than PP and LC.The total plate count and yeast and mould count of pork sausages inoculated with different lactic acid bacteria, were significantly (P>0.01) higher whereas the *Coliform* count and the *Salmonella count* were significantly (P>0.01) lower than the control after fermentation. Significantly (P>0.01) higher total plate counts, yeast and mould counts and lower *coliform* and *Salmonella counts* were observed in sausages treated with LP followed by PP and LC during fermentation and drying process.

Key words: *Pork sausages, fermentation, starter cultures, drying.*

INTRODUCTION

In view of globalization, food habits and job pattern of consumers have changed and a great demand for the higher proportion of processed meat products is being witnessed in recent years. A high microbial contamination can be expected in the meat that has been handled by the butchers in the slaughter houses or in the retail meat markets. In recent years addition of chemical preservatives has been restricted and the present trend is towards natural antimicrobial substances or biopreservatives (Sagdic *et al.*, 2003) for the preservation of meat and meat products. Thus natural biological acidification would be the method of choice for production of low pH meat products by fermentation. It preserves and enriches food, improves digestibility, and enhances the taste and flavor of foods. Furthermore, fermentation has the potential of enhancing food safety by controlling the growth and multiplication of number of pathogens in foods. Fermentation of meat is a means of preservation and the use of various microbial cultures, as a preservative appears to be more promising for India and other developing countries having hot climatic conditions and inadequate refrigeration facilities. Some Lactic Acid Bacteria (LAB) shows special promise, as they do not pose any health risk to man and are able to prevent the outgrowth of undesirable bacteria and opportunistic pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. Microbial antagonism is due to the production of metabolites such as lactic acid, acetic acid, diacetyl, hydrogen peroxide, and bacteriocins. Dry and Semi- dry fermented sausages are suitable carriers for probiotics into

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human gastrointestinal tract. Hence, the present study attempts to get an insight into the technology and microbiology of semi-dry sausage, fermentated with probiotic LAB to know the effect of different types of starter cultures.

MATERIALS AND METHODS

Large white Yorkshire pigs of above 80 Kg were obtained from 'Self Supporting Livestock Products Project' of Department of Livestock Products Technology, NTR College of Veterinary Science, Gannavaram. After deboning the carcass, less valuable parts of the carcass and organ meats were utilized for the study. Hot dressed carcass of pig was deboned manually within one hour of slaughter. Low valuable parts of carcass, bone scrapings, tongue, and heart were utilized and packed in LDPE bags and stored at 10 ºC for 24 hours. The meat was cut into small cubes of about 2 cm size. The cubes of above meats were minced twice using Meat Mincer (Model TC12E, Italy) with a plate hole diameter of 5 mm to obtain uniform mix. The freeze dried starter cultures of three types of Lactic Acid Bacteria (LAB), *Lactobacillus casei* (LC), *Lactobacillus plantarum* (LP) and *Pediococcus pentosaceus* (PP) were received in ampoules from National Collection of Dairy culture (NCDC), Dairy Microbiology Division, National Dairy Research Institute, Karnal -132 001. The mother cultures were prepared and stored at 4ºC. These cultures were sub cultured periodically checked for purity, morphology and biochemical characteristics at every 15 days. The concentration of bacteria was determined by total plate count method. The working bacterial suspension containing 10^8 CFU /ml was obtained by adjusting the dilution with normal saline solution. To the minced meat common salt and sodium nitrite were added and mixed for 1-2 minutes. Vegetable oil of 5% was added and the content was again mixed for 1 minute. This was followed by addition of condiment mix, spice mix, refined wheat flour and mixed for 1-2 minutes. Glucose and starter culture were added to the above mixture and mixed for 0.5 to 1 minute (Table 2). Sufficient care was taken to keep the end point temperature below 10 ºC and the emulsion was kept in refrigerator for 20-30 minutes. Sausage mix was stuffed into casings with manual sausage stuffer. The raw sausages were linked at about 6.5 cm apart to make sausages of uniform length. The sausages were washed with chilled water after linking and allowed 1-2 minutes for draining. These sausages were subjected for fermentation and drying (ripening) in fabricated fermentation chamber and fermentation was carried out at temperature of 25-27 ºC and relative humidity of 90 ±5 % for 12-14 hours followed by drying at 6-8 ºC at relative humidity 70 ± 5 % 8 days to obtain semi dry fermented pork sausages

Formulation for fermented sausages using pork

Ingredients	Percent
Meat (Pork)	74
Refined wheat flour	10
Vegetable fat	5
Ginger + garlic (2:1)	4
Cloves	0.5
Cinnamon	1
Cardamom	0.5
Pepper	1
Salt	2
Nitrite	0.005
Dextrose	1
Culture	1

The physico-chemical and microbiological characteristics were evaluated at 0 hour of sausage filling (raw sausages), after 12-14 hours of fermentation (fermented sausages) and after 8 days of drying (dried sausages) process of fermented meat sausages using different cultures.

Types of fermentation groups with pork were

C : Fermentation without starter culture

PLC :Fermentation of pork sausages with *Lactobacillus casei* ($1X10^6$cfu/g)

PLP : Fermentation of pork sausages with *Lactobacillus plantarum* ($1X10^6$cfu/g)

PPP : Fermentation of pork sausages with *Pediococcus pentosaceus* ($1X10^6$cfu/g)

The percent moisture (oven drying), percent protein (Kjeldhal method) and percent fat (using Soxhelet's apparatus) were estimated as per the procedures of AOAC (1995).The pH of the sample was determined by the procedure of Keller *et al* (1974). The pH was recorded by immersing the combined glass electrode of digital pH meter (Model: 101 E Deluxe pH meter) in the homogenate. The water holding capacity of the sample was determined by the procedure of Wierbicki *et al*., (1962). The water activity of samples were determined by using the formula developed by Lerici *et al.* (1983). The emulsion stability of the samples was determined by the method followed by Baliga and Madaiah (1970). Percent cooking yield was estimated by recording the difference between the pre and post cooking weights and expressed in percentages for shrinkage. Loss of weight of samples during storage was calculated by difference in initial and stored weight and expressed as percentage.

The total plate count per gram of samples were estimated as per the procedure recommended by Chestnut *et al.* (1977). For estimation of yeast and moulds the procedure used for estimation of total plate count was adopted except that Sabourads Dextrose agar was used in place of Standard Plate Count agar. Mac Conkey agar was used for Coli form counts where as Brilliant green agar was used for Salmonella counts. The data obatained was subjected for statistical analysis as per the SPSS (Version 10.0) software.

RESULTS AND DISCUSSION

The results of this study revealed that the per cent moisture of pork sausages inoculated with different lactic acid bacteria was significantly (P>0.01) lower, whereas protein and fat are higher than control(Table 1). Among treatments significantly (P>0.01) lower per cent moisture values and higher protein and fat were observed in pork sausages treated with LP than PP and LC. Per cent moisture decreased significantly (P>0.01), but protein and fat were increased during fermentation and drying process irrespective of culture (Table 2). The decreased moisture content of semi-dry fermented sausages might be due to reaching of isoelectric point of meat proteins during fermentation, which aids in moisture removal during drying phase (Klement *et al.* 1974). The results were in accordance with Steibing and Rodel (1988) who have recorded continuous reduction in moisture content during ripening period and Mukherjee *et al.* (2006) in fermented goat meat sausages. Similarly Kirupasankar (2006)

Table 1: Moisture, protein and fat content in pork sausages as influenced by different starter cultures during fermentation and drying Mean ± SE

Parameter during processing	PORK			
	C	LC	LP	PP
Moisture % before fermentation	60.09^{a1}±0.031	59.18^{b1}±0.008	59.16^{c1}±0.017	59.92^{b1}±0.007
After fermentation	59.75^{a2}±0.007	56.15^{b2}±0.013	54.16^{d2}±0.013	55.93^{c2}±0.009
After drying	58.14^{a3}±0.012	51.93^{b3}±0.012	47.17^{d3}±0.011	49.74^{b3}±0.014
Protein % before fermentation	17.55^{a3}±0.012	16.55^{c3}±0.006	17.54^{a3}±0.009	16.95^{b3}±0.015
After fermentation	18.65^{c2}±0.013	18.32^{d2}±0.006	19.14^{a2}±0.007	18.84^{b2}±0.011
After drying	19.94^{a1}±0.014	19.84^{b1}±0.009	19.85^{b1}±0.014	19.45^{c1}±0.015
Fat% before fermentation	22.24^{a3}±0.008	22.13^{b3}±0.009	22.15^{b3}±0.008	22.05^{c3}±0.010
After fermentation	22.53^{d2}±0.011	23.34^{c2}±0.009	23.65^{a2}±0.013	23.44^{b2}±0.008
After drying	23.63^{d1}±0.009	24.06^{c1}±0.018	24.74^{a1}±0.009	24.45^{b1}±0.010

C-Control, LC-*Lactobacillus casei,* LP-*Lactobacillus plantarum* and PP-*Pediococcus pentosus*

Mean values between cultures with different alphabetical superscripts differ significantly (P< 0.01)

Mean values between storage periods with different numerical superscripts differ significantly (P< 0.01)

observed decrease in moisture and increase in protein and fat content of fermented sausages after fermentation and drying. The increase in protein and fat may be due to corresponding moisture loss during drying process in treatments than in control. The per cent fat increased significantly (P>0.01) during fermentation and drying may be due to corresponding moisture loss during drying process in treatments than in control.

The pH, WHC, water activity, cooking yield of pork sausages inoculated with different lactic acid bacteria were significantly (P>0.01) lower where as shrinkage and emulsion stability values were higher than the control (Table 2). Among treatments significantly (P>0.01) lower pH,WHC,water activity, cooking yield values but higher shrinkage and emulsion stability values were observed in pork sausages treated with LP when compared to PP and LC (Table 3). The pH decreased significantly (P>0.01) during fermentation and drying process by more than 1.0 unit irrespective of culture might be due to the production of lactic acid, which brings down the pH to a level characteristic of fermented sausages (Everson *et al*. 1970). The decrease in water holding capacity during fermentation and drying may be due to significant decrease in pH by lactic acid starter cultures. The decrease in water activity by 0.03 units in semi-dry fermented sausages might be due to decreased moisture content of the sausages during ripening process.The results of this study were in agreement with Stiebing and Rodel (1988) who reported reduced a_w after seven days of drying and speed of reduction was significantly affected by moisture gradient during drying period of sausages. The emulsion stability increased significantly (P>0.01) during fermentation and drying process might be due to pH reduction and

Table 2: Mean ± SE of pH,WHC,Water activity,Emulsion stability,Cooking yield and shrinkage in pork sausages as influenced by different starter cultures during fermentation and drying

Parameter	During Processing	PORK			
		C	LC	LP	PP
pH	Before fermentation	5.43^{a1} ±0.008	5.42^{a1} ±0.005	4.95^{c1}±0.012	5.25^{b1}±0.004
	After Fermentation	4.45^{a2}±0.007	4.25^{b2}±0.008	3.94^{c2}±0.013	3.93^{c2}±0.009
	After Drying	4.42^{a2}±0.010	4.14^{b3}±0.014	3.82^{c3}±0.007	3.84c3±0.016
WHC	Before fermentation	57.14^{a1}±0.009	57.15^{a1}±0.013	56.93^{b1}±0.012	56.35^{c1}±0.007
	After Fermentation	56.14^{a2}±0.007	55.95^{b2}±0.013	53.14^{d2}±0.014	54.14^{c2}±0.011
	After Drying	55.94^{a3}±0.012	53.16^{b3}±0.014	51.15^{d3}±0.014	52.64^{c3}±0.010
Water Activity	Before fermentation	0.962^{a1}±0.0005	0.962^{a1}±0.0005	0.961^{a1}±0.0005	0.961^{a1}±0.0008
	After Fermentation	0.952^{a2}±0.0006	0.937^{b2}±0.0007	0.925^{c2}±0.0008	0.934^{b2}±0.0010
	After Drying	0.934^{a3}±0.001	0.923^{b3}±0.0008	0.891^{d3}±0.0006	0.913^{c3}±0.0009
Emulsion stability	Before fermentation	6.93^{a1}±0.009	6.97^{a1}±0.006	6.94^{a1}±0.006	6.97^{a1}±0.006
	After Fermentation	6.85^{ab2}±0.017	6.88^{a2}±0.038	6.57^{c2}±0.008	6.81^{b2}±0.005
	After Drying	6.16^{a3}±0.009	5.74^{b3}±0.013	5.33^{d3}±0.008	5.46^{c3}±0.008
Cooking yield %	Before fermentation	93.96^{a1}±0.014	93.73^{b1}±0.014	93.17^{c1}±0.01	93.13^{c1}±0.007
	After Fermentation	92.74^{a2}±0.007	90.05^{b2}±0.012	88.13^{d2}±0.006	89.15^{c2}±0.009
	After Drying	91.13^{a3}±0.008	91.13^{b3}±0.008	86.02^{d3}±0.006	87.03^{c3}±0.006
Shrinkage %	Before fermentation	0.52^{a3}±0.007	0.52^{a3}±0.007	0.52^{a3}±0.006	0.51^{a3}±0.007
	After Fermentation	0.94^{d2}±0.013	6.92^{c2}±0.009	7.33^{a2}±0.006	7.16^{b2}±0.011
	After Drying	3.63^{d1}±0.012	16.06^{c1}0±.012	17.35^{a1}±0.011	16.63^{b1}±0.009

C- Control,LC- Lactobacillus Casei,LP-Lactobacillus plantarum and PP-Pediococcus pentosus

Mean values between cultures with different alphabetical superscripts differ significantly (P< 0.01)

Mean values between storage periods with different numerical superscripts differ significantly (P< 0.01)

Table 3 : Mean ± SE of Total plate count, *Coliform* count, Salmonella and Yeast and mould count (log CFU/g) in pork sausages as influenced by different starter cultures during fermentation and drying

Parameter	During Processing	PORK			
		C	LC	LP	PP
Total plate count	Before fermentation	2.531^{b3}±0.0004	3.572^{a3}±0.0005	3.574^{a3}±0.0004	3.572^{a3}±0.0007
	After Fermentation	3.352^{d2}±0.0005	5.285^{c2}±0.001	5.432^{a2}±0.0008	5.363^{b2}±0.0009
	After Drying	4.393^{d1}±0.001	4.294^{c1}±0.0008	4.422^{a1}±0.0009	4.372^{b1}±0.0004
Coli form count	Before fermentation	3.203^{a3}±0.0007	3.113^{c1}±0.0009	2.105^{d1}±0.0007	3.122^{b1}±0.001
	After Fermentation	3.613^{a2}±0.0009	2.612^{b2}±0.0006	2.421^{d2}±0.0005	2.532^{c2}±0.0006
	After Drying	3.812^{a1}±0.0006	1.542^{b3}±0.0007	1.353^{d3}±0.0007	1.412^{c3}±0.0007
Salmonella count	Before fermentation	0.923^{a3}±0.0008	0.913^{b1}±0.0007	0.862^{d1}±0.0006	0.873^{c1}±0.0006
	After Fermentation	0.932^{a2}±0.0008	0.823^{b2}±0.0006	0.682^{d2}±0.001	0.751^{c2}±0.0008
	After Drying	1.871^{a1}±0.0006	0.705^{b3}±0.001	0.565^{d3}±0.001	0.675^{c3}±0.001
Yeast and mould	Before fermentation	1.682^{a3}±0.0008	1.684^{a3}±0.001	1.612^{b3}±0.0006	1.606^{c3}±0.001
	After Fermentation	1.733^{d2}±0.0007	1.874^{c2}±0.001	1.934^{a2}±0.001	1.884^{b2}±0.001
count	After Drying	2.244^{d1}±0.0009	2.393^{c1}±0.0008	2.572^{a1}±0.0004	2.476^{b1}±0.001

C- Control, LC-*Lactobacillus casei,* LP-*Lactobacillus plantarum* and PP-*Pediococcus pentosus*
Mean values between cultures with different alphabetical superscripts differ significantly (P< 0.01)
Mean values between storage periods with different numerical superscripts differ significantly (P< 0.01)

moisture loss during fermentation and drying process. The results were in agreement with Hargus *et al*. (1970) and Thomas *et al*. (2006) in restructured buffalo meat nuggets. The reduction in cooking yield of sausages fermented with starter culture has been reported by Salahuddin (1992) and Kirupasankar (2006). The increase in shrinkage of fermented sausages is directly proportional to moisture loss during drying. Stiebing and Rodel (1988) stated that shrinkage in dry sausages during ripening was caused only by moisture evaporation from outer layer for first few days of drying and in later stages water migrated from core portion and evaporated through outer layer.

The total plate count and yeast and mould counts of pork sausages inoculated with different lactic acid bacteria, were significantly (P>0.01) higher where as *Coliforms* and *Salmonella* counts were lower than the control after fermentation (Table 3). The results were in accordance with Subsoontorn (1985) who reported that sausages containing only natural flora had a lower bacterial population than those containing starter culture during the first period of storage in Thuringer sausages. Significantly higher total plate counts and yeast and mould counts but lower *Coliforms* and *Salmonella* counts were observed in LP followed by PP and LC during drying process. The decrease in total plate count of semi-dry fermented sausages might be due to production of bacteriocin and bacteriocin like products that display anti-bacterial activity (De Vuyst and Vandamme, 1994) and due to decrease in water activity during ripening process. The reduction in *coliform* counts after fermentation and drying is due to lower pH, bacterial competition for nutrients and action of antimicrobial substances (bacteriocins) and metabolites produced by LAB (Brankova *et al.* 1984; Paneras and Bloukas, 1984; Ellajosyula *et al*. 1998), Drosinos *et al.* (2005) in fermented sausages, Hwang *et al*. (2009) in soudjouk-style fermented sausage. The yeast and mould count increased significantly (P>0.01) during fermentation and drying process irrespective of culture could be related to acidic environment produced by the starter cultures (Bacus, 1984).

CONCLUSION

The fermented sausages prepared under this study were acceptable physico-chemically and microbiologically during fermentation and drying with different lactic acid bacteria, LC, LP and PP than control. However pork sausages fermented with LP considered to be superior in respect to its quality characteristics that have lower pH, water activity and microbial counts followed by PP and LC.

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CIRCULAR FOR 5th ANNUAL GENERAL BODY MEETING

The 5th Annual General Body Meeting of Indian Meat Science Association will be held on 08.02.2012 at 4.00 p.m. in the venue of IMSACON-V at Hyderabad.

Agenda to be discussed:

1. Election / Selection of New Executive Committee.
2. Selection of New Editorial Board of Journal of Meat Science.
3. Presentation of Audited accounts of the Association.
4. Any other, with the permission of the chair.

Secretary
IMSA