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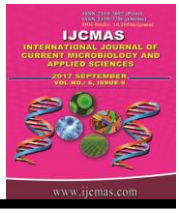

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Effect of Starter Cultures and Methods of Packaging on Quality Characteristics of Pork Ham

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A B S T R A C T

Wet cured pork hams inoculated with *Lactobacillus acidophilus* and *Micrococcus varians*$_{483}$ had higher total viable count, lactic acid bacteria and Micrococcaceae counts and lower Enterobacteriaceae and coliform counts. pH of the inoculated hams was lower. ERV, WHC and a_w decreased significantly with storage period. MAP was found to be better in maintaining reduced a_w during refrigeration storage of hams. Starter culture inoculated hams of 60th d of storage had significantly higher TVC, LAB and Micrococcaceae count and significantly lower Enterobacteriaceae and coliform counts. MAP lowered the TVC, LAB, Micrococcaceae and Enterobacteriaceae counts significantly whereas, the coliform counts were significantly lower in VP than the MAP samples. Starter culture inoculated hams were superior in terms of their sensory properties and those packaged under MAP were rated better than the VP samples.

Introduction

Starter cultures bring about unique and distinctive flavour, improved palatability, colour, tenderness, microbiological safety and a host of other desirable attributes to fermented meat products. Typical starter cultures used for fermentation of cured whole ham include lactic acid bacteria along with nitrite/nitrate reducing strains of Micrococcaceae to mainly contribute to stabilization of cured meat colour and aroma development by transforming the triglycerides into glycerol and fatty acids by their lipolytic enzymes. These acids are sequentially broken down into carbonyls producing a distinct aroma.

LAB lower pH of meat and produce bacteriocin to ensure biological stability of the product (Con and Gökalp, 2000; Yin and Jiang, 2001; Fadda *et al.*, 2002). Method of packaging has an important bearing on the quality and shelf stability of pork ham. Vacuum packaging or modified atmosphere packaging are being increasingly applied for ham distribution and retail sale (Stiles, 1990).

The objective of the present study is to elucidate the effects of the meat starters comprising of *Lactobacillus acidophilus* and *Micrococcus varians*$_{483}$ and the methods of packaging on the physico-chemical,

microbiological and sensory attributes of pork ham.

Materials and Methods

Curing of ham

Hams weighing 5-6 kg were fabricated out from pig carcass by separating the hind leg at the point of hip joint. A long deep incision was given on the medial aspect of the ham and the aitch bone was removed out. Deboned hams were wet cured with the help of a multi-needle brine injector (Model: PI 11, Gunther Machinenbau, Germany). About 1 to 1½ litres of brine per ham was injected (Common salt 5%, Brown sugar 1%, Sodium nitrite 0.025%, Sodium tripolyphosphate 0.5%, Sodium ascorbate 0.1% and Liquid smoke 1%). After brine injection, hams were vacuum tumbled at 100kPa for an hour in a vacuum tumbler (Model: LU 2x25, Lumar Ideal II, Inc, Canada).

Application of starter cultures

Meat starter cultures of *L. acidophilus* and *M. varians* M_{483} maintained in the department were grown in MRS (de Mann *et al.,* 1960) and Mannitol salt broth (Chapman, 1945), respectively at 37^0C for 18-20 h and were pelleted by centrifuging at 5000rpm for 10 min in a refrigerated centrifuge (Model: 3K30, Sigma, Germany). The pellets were resuspended in sterile physiological saline solution to the desired concentration of cells. Number of cells per millilitre was determined by direct microscopic count as described by Harrigan and McCance (1976). The mixed cultures were then injected at multiple sites of the ham to reach a dose level of approximately 10^6cfu/g.

Hams were then immersed in a weaker brine solution [common salt reduced by 0.5% (w/v)] and stored at 4^0C for 10 d. After this fermentation period, the rind and fat from the hams along with fascia were removed and kept hung in ham net bags for 15 min. Cold smoking at 25^0C was done for an hour in a smoke cabinet (Model: 1600 RET-C, Kerres, Germany). After smoking, the hams were stored at 12^0C for 4 d. Hams were then cooked to an internal temperature of 75^0C in a cooking vat (Model: Mera 200, Talsa, Spain) for an hour. Cooked hams were cooled for overnight at 4^0C before carving into slices and then modified atmosphere packaged and vacuum packaged in a vacuum packaging machine (Model: QS 500 MAP, Sevana, India). The gas mixture of CO_2, N_2 and O_2 in the MAP was in the ratio of 1: 1: 1.

Physico-chemical properties

pH of the ham samples were determined on 1st, 15, 30, 45, 50, 55 and 60th day of refrigeration storage by following the method described by Pippen *et al.,* (1965) by using a digital pH meter (Model: 780, Metrohm, Switzerland). Water holding capacity was determined at time intervals as in case of pH by following the 'filter press technique' described by Grau and Hamm (1953) and that of the extract release volume by following the 'folded filter paper' method described by Pearson (1967). The water activity of ham samples was determined indirectly by means of Equilibrium Relative Humidity as described by Labuza *et al.,* (1976).

Microbiological quality

Microbial counts of hams were done immediately after curing and tumbling by following the methods described by Harrigan and McCance (1976). Total viable count, LAB, Micrococcaeae and Enterobacteriaceae counts were done after inoculation at 24, 48 and 72 h and on the 10th d of fermentation. The microbiological quality of ham was again evaluated on 60th d of refrigeration storage.

Sensory evaluation

Hams stored for 60th day were cut into small pieces and warmed up by light frying and served to a 7-membered semi trained panel for sensory evaluation using the 9-point hedonic score card as described by Bratzler (1971).

Statistical analysis

The data of the study were analyzed statistically as per SAS Enterprise Guide 4.2.

Results and Discussion

Physico-chemical properties

Starter culture inoculated hams packaged under VP had lower pH values than the MAP samples (Table 1). Increasing concentration of CO_2 gave rise to lowering of pH due to the absorption of CO_2 by dry-cured ham resulting in the production of carbonic acid (Dixon and Kell, 1989 and Juncher *et al.,* 2001). Cilla *et al.,* (2006) observed that pH of the dry cured MAP hams (20% CO_2 + 80% N_2) were significantly lower than the VP samples throughout the storage period. However, Jin *et al.,* (2010) did not find any significant difference in pH values between packaging methods (VP, MAP and nitrogen packaging) during storage except at 60 d of storage.

The ERV of hams packaged under MAP and VP systems gradually decreased along with storage. With an initial value of 34.80 ± 0.25 and 41.40 ± 0.43 ml in the control and the treated samples, respectively, the ERV gradually decreased to 18.07 ± 0.58 and 22.00 ± 0.41ml on 60th d showing significant difference amongst the control, MAP and VP samples. Strange *et al.,* (1977) while evaluating seven rapid analytical tests to monitor alterations in meat quality during storage opined that the ERV could not predict or monitor meat quality as expected. The treated samples showed higher ERV than the control ones. The VP samples of both the treated and the control groups maintained a higher ERV than the MAP samples throughout the storage period.

From the initial mean level of 1.23 ± 0.04 and 1.58 ± 0.07 cm^2 in the control and the treated groups, respectively, WHC decreased to 2.92 ± 0.04 and 3.03 ± 0.04 cm^2 in the MAP and VP systems of the control group and to 2.86 ± 0.03 and 3.16 ± 0.02 cm^2 in the treated group, respectively on the 60th d. Boschkova *et al.,* (1983) in their study on the influence of starter cultures upon the hydrophilic properties of non-comminuted raw dried pork products observed that the WHC decreased with the drop in pH values of the meat mass. The VP ham showed lower WHC than the MAP in both the treated and the control groups.

The control and the treated hams had mean a_w values of 0.91 ± 0.00 and 0.89 ± 0.01, respectively on the first day which continued to decline reaching the final mean a_w of 0.81 ± 0.01 and0.80 ± 0.00 in the MAP control and treated samples, respectively and 0.83 ± 0.01 and 0.81 ± 0.00 in the VP of the control and treated samples, respectively on the 60th d of the storage period. The a_w of the control group was higher than the treated group. MAP was found to be a better packaging system in maintaining reduced a_w of the hams during refrigeration storage. Scannell *et al.,* (2002) reported that in a novel cooked fermented ham, the a_w of both control and treated groups ranged between 0.97 and 0.98 throughout the storage period.

Total viable count

The inoculated hams showed an increase in the TVC from 24 h of inoculation to 72 h and then gradually decreased till the 10th d of fermentation (Table 2) whereas, the TVC grew till the 10th d in the control samples.

Higher TVC of the treated samples was due to the added starter cultures. The TVC of the control ham packaged under MAP and VP were found to be lower than the treated hams on the 60^{th} d of storage. Also, the VP ham exhibited higher TVC than the samples packaged under MAP in both groups (Table 3). Kotzekidou and Bloukas (1996) reported that the average of total plate count was lower in control hams than in hams added with protective cultures (*L. alimentarius* and *Staphylococcus xylosus*). Scannell *et al.*, (2004) reported that while the control samples maintained the level of 10^4cfu/g, starter culture inoculated fermented non-dried whole muscle ham had mean mesophilic aerobic counts of 10^8cfu/g.

LAB and Micrococcaceae count

The inoculated ham showed a rise in the lactobacilli and Micrococcaceae count from 24 h of inoculation to 72 h and then gradually decreased till the 10^{th} d of fermentation. Similar trends were also observed in the control ham but the counts were lower than the inoculated ones.

The LAB and Micrococcaceae count of the control hams stored under MAP and VP for 60 d at 4^0C were found to be lower than the treated hams (Table 3). It was noticed that the VP hams exhibited higher lactobacilli counts than the MAP samples but as regards to Micrococcaceae, the MAP hams exhibited higher counts than the samples packaged under VP.

Scannell *et al.*, (2002) in their study on a novel cooked ham product reported that the number of LAB increased from 10^7 to approximately 10^9cfu/g after 3 d of fermentation at both 12 and 18^0C. After 7 d of fermentation, LAB counts in ham fermented at 18^0C were considerably higher than those fermented at 12^0C. Studies on the shelf-life analysis of sliced ham revealed that the unfermented hams had reached 10^7/g LAB within 21 d of refrigeration storage whereas, the fermented hams had not reached this cut off point even after 56 d indicating an increased shelf-life in terms of microbiological stability.

Scannell *et al.*, (2004) reported that hams inoculated with *M. varians* had an initial Micrococcaceae count of approximately 10^7cfu/g. In keeping with trends observed in other fermented meats (Coventry and Hickey, 1991; Garcia *et al.*, 1992), these levels remained relatively constant throughout the fermentation process except when GDL was combined with *M. varians*, their numbers decreased considerably.

Enterobacteriaceae count

The mean Enterobacteriaceae count of the control and treated hams showed a fluctuating pattern throughout the fermentation period. The substrate and the packaging are the two factors which affect the growth of spoilage flora in meat and meat products. The Enterobacteriaceae count of the control ham was higher than the treated samples on the 60^{th} d of storage. The VP ham showed higher counts than the MAP samples.

Liebetrau and Grossmann (1976) reported that application of lactobacilli in the production of fermented meat products effectively reduced the presence of Enterobacteriaceae and enterococci in the finished product.

Scannell *et al.*, (2002) reported that *Listeria*, *Staph. aureus* and *Salmonella* were not detected in the hams inoculated with *L. sakei* and *Staph. carnosus* as well as in the non-inoculated hams over the 7 d fermentation period.

Table.1 Changes in the physico-chemical properties of ham stored at 4ºC under different packaging systems (comparison of means of groups according to days within packaging and means of packaging according to days within groups)

Parameter	Groups	Storage period (d)											
		15		30		45		50		55		60	
		Packaging system											
		MAP	VP	MAP	VP	MAP	VP	MAP	VP	MAP	VP	MAP	VP
pH	Control	6.12 ± 0.04	6.06 ± 0.03	6.05 ± 0.04	6.03 ± 0.05	5.94 ± 0.04	5.89 ± 0.04	5.79 ± 0.02	5.73 ± 0.04	5.62 ± 0.02	5.58 ± 0.03	5.51^{A} ± 0.02	5.41^{B} ± 0.03
	Treated	6.01 ± 0.09	5.94 ± 0.08	5.97 ± 0.08	5.92 ± 0.07	5.86 ± 0.06	5.78 ± 0.05	5.73 ± 0.05	5.63 ± 0.05	5.60 ± 0.05	5.46 ± 0.05	5.44 ± 0.05	5.29 ± 0.05
ERV (ml)	Control	30.97^{a} ± 0.90	34.30^{a} ± 1.44	30.53^{A} ± 0.93	34.00^{aB} ± 0.76	28.1^{A} ± 0.64	31.50^{B} ± 0.56	22.67^{aA} ± 1.19	27.04^{aB} ± 1.12	21.43^{aA} ± 0.77	24.47^{aB} ± 0.90	18.07^{A} ± 0.58	22.00^{B} ± 0.41
	Treated	37.73^{bA} ± 0.45	41.13^{bB} ± 0.50	31.4^{A} ± 0.07	36.73^{bB} ± 0.33	30.37 ± 0.93	33.03 ± 0.94	30.13^{b} ± 1.59	32.27^{b} ± 0.53	29.90^{b} ± 0.48	30.63^{b} ± 0.35	21.70 ± 1.97	24.67 ± 1.88
WHC (cm^2)	Control	1.30^{a} ± 0.02	1.36^{a} ± 0.03	1.45^{a} ± 0.03	1.55^{a} ± 0.04	1.70^{a} ± 0.07	1.79^{a} ± 0.06	2.00^{a} ± 0.06	2.02^{a} ± 0.05	2.30^{A} ± 0.07	2.58^{aB} ± 0.04	2.92 ± 0.04	3.03^{a} ± 0.04
	Treated	1.62^{bA} ± 0.03	1.81^{bB} ± 0.06	1.7^{bA} ± 0.03	1.90^{bB} ± 0.07	2.03^{bA} ± 0.03	2.24^{bB} ± 0.07	2.2^{b} ± 0.04	2.34^{b} ± 0.07	2.53^{A} ± 0.07	2.78^{bB} ± 0.07	2.86^{A} ± 0.03	3.16^{bB} ± 0.02
a_w	Control	0.90^{aA} ± 0.00	0.91^{aB} ± 0.00	0.88 ± 0.00	0.88 ± 0.01	0.88^{a} ± 0.00	0.88 ± 0.00	0.86^{aA} ± 0.00	0.88^{aB} ± 0.00	0.83^{A} ± 0.01	0.85^{B} ± 0.00	0.81^{A} ± 0.01	0.83^{B} ± 0.01
	Treated	0.87^{bA} ± 0.00	0.89^{bB} ± 0.00	0.87 ± 0.01	0.88 ± 0.01	0.85^{b} ± 0.01	0.85 ± 0.02	0.82^{b} ± 0.01	0.84^{b} ± 0.01	0.82 ± 0.01	0.84 ± 0.01	0.80^{A} ± 0.00	0.81^{B} ± 0.00

n=5,
Means in a column bearing uncommon superscript within each packaging (lower case) differ significantly.
Means in a row bearing uncommon superscript within each treatment group (uppercase) differ significantly.

Table.2 Microbiological quality of ham (comparison of means between treatment groups and between storage periods within each treatment group)

Microbiological Parameters	Before inoculation	Storage period							
		24h		48h		72h		10d	
		Groups							
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
TVC (log_{10}cfu/g)	4.19 ± 0.03	4.95^{aA} ± 0.01	8.17^{bA} ± 0.01	5.06^{aB} ± 0.01	8.39^{bB} ± 0.01	5.13^{aC} ± 0.01	8.60^{bC} ± 0.02	5.48^{aD} ± 0.03	7.75^{bD} ± 0.02
LAB (log_{10}cfu/g)	1.94 ± 0.07	2.73^{aA} ± 0.02	7.05^{bA} ± 0.03	3.11^{aB} ± 0.01	7.77^{bB} ± 0.04	3.37^{aC} ± 0.01	8.47^{bC} ± 0.01	3.01^{aB} ± 0.01	8.43^{bC} ± 0.05
Micrococcaceae (log_{10}cfu/g)	1.66 ± 0.11	2.40^{aA} ± 0.01	6.73^{bA} ± 0.02	2.88^{aB} ± 0.01	7.29^{bB} ± 0.01	3.15^{aC} ± 0.02	7.82^{bC} ± 0.01	2.80^{aD} ± 0.02	6.45^{bD} ± 0.01
Enterobacteriaceae (log_{10}cfu/g)	4.02 ± 0.02	3.51^{aA} ± 0.01	2.07^{bA} ± 0.01	4.81^{aB} ± 0.02	3.40^{bB} ± 0.01	4.81^{aB} ± 0.01	2.82^{bC} ± 0.02	5.01^{aC} ± 0.02	2.11^{bA} ± 0.01
Coliforms (MPN/g)	375 ± 49.07	120^{aA} ± 0.00	64^{bA} ± 0.00	95^{aB} ± 0.00	53^{bB} ± 0.00	93^{aC} ± 0.00	29^{bC} ± 0.00	44^{aD} ± 0.00	23^{bD} ± 0.00

n=5

Means in a row bearing uncommon superscript (lower case) between groups within each period differ significantly.

Means in a row bearing uncommon superscript (upper case) between different periods within each group differ significantly.

Table.3 Microbiological quality of ham stored for 60 days at 4ºC under different packaging systems (comparison of means of groups within packaging and means of packaging within groups)

Parameter	Packaging Methods	Groups	
		Control	Treated
TVC (log_{10}cfu/g)	MAP	5.15^{bA} ± 0.04	7.31^{aA} ± 0.02
	VP	5.74^{bB} ± 0.05	7.84^{aB} ± 0.05
LAB (log_{10}cfu/g)	MAP	4.11^{bA} ± 0.01	6.94^{aA} ± 0.03
	VP	4.61^{bB} ± 0.02	7.08^{aB} ± 0.01
Micrococcaceae (log_{10}cfu/g)	MAP	3.99^{b} ± 0.03	6.16^{aA} ± 0.02
	VP	3.82^{b} ± 0.09	6.03^{aB} ± 0.03
Enterobacteriaceae (log_{10}cfu/g)	MAP	2.94^{aA} ± 0.03	2.25^{bA} ± 0.04
	VP	3.07^{aB} ± 0.03	2.57^{bB} ± 0.05
Coliforms (MPN/g)	MAP	64^{aA} ± 0.00	29^{bA} ± 0.00
	VP	29^{aB} ± 0.00	23^{bB} ± 0.00

n=5

Means in a row bearing uncommon superscript (lower case) between groups within each packaging system differ significantly

Means in a column bearing uncommon superscript (upper case) between packaging within each group differ significantly.

Table.4 Sensory properties of ham (comparison of means of groups within each packaging system and means of packaging system within each group)

Sensory Attributes	Treatment Groups	Packaging System	
		MAP	VP
Appearance	Control	$7.07^{a} \pm 0.04$	$7.03^{a} \pm 0.03$
	Treated	$8.71^{b} \pm 0.06$	$8.36^{b} \pm 0.21$
Colour	Control	$7.00^{a} \pm 0.00$	$6.96^{a} \pm 0.03$
	Treated	$8.17^{b} \pm 0.07$	$8.07^{b} \pm 0.04$
Taste	Control	$6.93^{a} \pm 0.04$	$7.82^{a} \pm 0.07$
	Treated	$8.21^{b} \pm 0.18$	$6.78^{b} \pm 0.11$
Tenderness	Control	$7.31^{a} \pm 0.12$	$7.17^{a} \pm 0.07$
	Treated	$7.89^{b} \pm 0.07$	$7.71^{b} \pm 0.08$
Flavour	Control	$6.46^{aA} \pm 0.12$	$6.07^{aB} \pm 0.04$
	Treated	$7.89^{bA} \pm 0.09$	$7.53^{bB} \pm 0.09$
Juiciness	Control	$6.17^{aA} \pm 0.03$	$5.96^{aB} \pm 0.03$
	Treated	$8.18^{b} \pm 0.09$	$7.89^{b} \pm 0.09$
Overall acceptability	Control	$6.82^{aA} \pm 0.04$	$6.66^{aB} \pm 0.03$
	Treated	$8.05^{b} \pm 0.05$	$7.97^{b} \pm 0.04$

n=5

Means in a column bearing uncommon superscripts (lowercase) differ significantly between groups within each packaging system of a trait

Means in a row bearing uncommon superscript (uppercase) differ significantly between packaging systems within each treatment group of a trait.

Kotzekidou and Bloukas (1996) reported that the growth of pseudomonads was exponential during the first 2 weeks of storage of hams produced by the addition of protective cultures and reached 10^{4}cfu/g. The highest population of pseudomonads was observed in cooked hams produced by *L. alimentarius*. They further reported that in both the treated and the control vacuum packed cooked ham, the growth of Enterobacteriaceae and enterococci was very slow at 4ºC.

Coliform count

There was a gradual reduction in the number of coliform organisms in the ham after 24 h of inoculation. The coliform numbers started diminishing markedly in both the treated and the control samples during the fermentation period. It was observed that the inoculated samples had a lower coliform count than the control samples which could be attributed to the antagonism by the starter organisms.

The mean coliform count of the treated ham packaged under MAP and VP were found to be lower than the control samples on the 60th d of storage. It was also noticed that MAP ham exhibited higher counts than VP samples in both the treated and the control groups.

Scannell *et al.,* (2002) reported that coliform counts were not detected in the hams inoculated with *L. sakei* and *Staph. carnosus* as well as in the non-inoculated hams over the 7 d fermentation period.

Jin *et al.,* (2010) in their study on the effects of packaging methods and refrigerated storage on the quality of dry-cured pork neck reported lower coliform counts in MAP than in the VP samples at 60 and 90 d of storage.

Sensory evaluation of ham

The results on sensory evaluation of the ham indicated that use of the mixed starter cultures brought about desirable changes with regards to all the traits studied (Table 4). Hams packaged under MAP were preferred by the panellists over the VP in either of the groups for all the traits except for taste of the control sample. Better panel preference of the starter culture treated ham could be attributed to the production of carbonyl compounds, volatile fatty acids, lactic acid, diacetyl, acetoine etc. by the starter cultures that contributed to the taste and flavour of the finished product.

Scannell *et al.,* (2002) reported better panel ratings of hams fermented with *L. sakei* and *Staph. carnosus* for taste and texture profile and were more acceptable than control hams. Sanchez-Molinero and Arnau (2008) reported that there was a decrease in sweetness in hams inoculated with a commercial starter culture containing LAB, Gram- positive catalase-positive cocci and yeasts which could be due to the fermentation of dextrose by LAB as suggested by the lowered pH values. Kotzekidou and Bloukas (1996) reported that the sliced vacuum packed hams produced with protective cultures had better odour and taste scores than the controls.

Wet cured pork hams were inoculated with *Lactobacillus acidophilus* and *Micrococcus varians*$_{483}$ at 10^6cfu/g. Starter culture inoculated hams had higher total viable count, lactic acid bacteria and Micrococcaceae counts and lower Enterobacteriaceae and coliform counts. pH of the inoculated hams was lower and VP hams had lower pH value than the MAP samples. ERV, WHC and a_w decreased significantly with storage period. MAP was found to be a better method of packaging than the VP in maintaining reduced a_w of hams during refrigeration storage. Treated ham samples evaluated on the 60th d had significantly higher TVC, LAB and Micrococcaceae count and significantly lower Enterobacteriaceae and coliform counts. MAP lowered the TVC, LAB, Micrococcaceae and Enterobacteriaceae counts significantly whereas, the coliform counts were significantly lower in VP than the MAP samples. Starter culture inoculated hams were rated superior in terms of their sensory properties whereas, hams packaged under MAP were rated superior for sensory properties than those packaged under VP.

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