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Effect of Inoculum Levels and Sugar Concentrations on the Preparation of Fermented Semi-dry Fish Sausages

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Fermented semi-dry fish sausages were prepared with different levels of lactic acid (LAB) inoculum (10^6/g, 10^7/g and 10^8/g) and sugar (1.0% to 5.0%) and subjected to chemical and organoleptic evaluation. Higher levels of LAB inoculum resulted in faster fermentation rate. Growth of LAB decreased at higher rates of LAB inoculum. At lower levels of LAB inoculum, TPC, TMA and TVB values were higher. There was no significant difference among the different levels of LAB inoculum with respect to organoleptic quality, but lower scores were received for the high inoculum level. Fermentation rate was not affected by the different levels of sugar and 1% sugar level was preferred by the panelists.

A rapid lactic acid bacterial (LAB) growth in the initial phase of fermentation of the sausages is necessary so as to suppress the simultaneous growth of the spoilers and pathogens. The fermentation rate is influenced by the level of LAB inoculum and sugar among other factors.

As a part of standardisation of semi-dry fish sausages, different levels of LAB inoculum and sugar were tried in the present study to find out a suitable level for the Indian palate and their effects on the fermentation pattern.

Materials and Methods

Good quality croaker (*Johnius* sp.) was brought from the local market in iced condition, dressed, washed with 5-10 ppm chlorine water, rinsed, fish meat separated, frozen and stored until further use. Before use the meat was thawed and minced. *Pediococcus acidilactici* (NCL, Poona) was used as the starter culture.

Fish mince was mixed with the sausage ingredients (Table 1); as per the recipe of Kramlich *et al.* (1973) and Bawa & Delong (1985) with slight modifications and replacing animal meat with fish meat. The level and composition of spice mixture and salt were as per Joshi (1990). The order of addition of ingredients was: Fish mince, sugar, spices, colour, fat, salt and young LAB cultures. Sausages were prepared with 3 levels of LAB inoculum namely, 2%, 0.2% and 0.02% of the sausage mix which was equivalent to 10^8, 10^7 and 10^6cfu/g of sausage mix, respectively.

For the purpose of standardisation of sugar, the 2% level of LAB inoculum was used and sausages were prepared with 1, 2, 3, 4 and 5% concentration of sugar. After mixing for a brief period of 5-10 min in a stainless steel vessel using a ladle, the sausage mix was stuffed into the natural casing prepared as per Madhwaraj *et al.* (1980). The sausages were linked at 50-75mm intervals, washed and hung in the smoking chamber at room temperature for fermentation till the desired pH of 5.0-5.1 was reached. These were smoked at 35 to 40°C for 2 h till a pH of 4.6 to 4.75 was reached (Kramlich *et al.*, 1973 and Christians, 1980).

The pH and total titratable acidity (TTA) as per AOAC (1975); Trimethyl amine

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Nitrogen(TMA-N) and total volatile bases (TVB) by Convay microdiffusion, sugar content as per Winton & Winton (1958), lactic acid bacterial (LAB) count and total plate count (TPC) as per Harrigan & Mc Cance (1976) of the sausages were analysed and organoleptic evaluation of the sausages was done by a group of ten trained panelists using a 10 point hedonic scale to arrive at an optimum level of LAB inoculum and sugar.

Table 1. *Recipe of semi dry sausage mix used for standardisation*

Ingredients	% of ingredients for standardisation of	
	LAB culture	Sugar
Fish	86.48	87.5
Hydrogenated veg. fat	4.0	4.0
Spice mixture*	2.5	2.5
Salt	3.0	3.0
Lab cultures	0.02,0.2 & 2.0	2.0
Sugar	4.0	1,2,3, 4 & 5
*Spices mixture composition		
Black pepper	0.35	
Chilli powder	0.45	
Garlic	0.10	
Ginger	0.10	
Coriander	0.05	

Results and Discussion

The results indicated that higher the level of inoculum, faster was the fermentation rate (Table 2). The fermentation time required to attain a pH of 5.1 in the sausage was 10 h, 17 h and 22 h when the rate of inoculum was 10^8/g, 10^7/g and 10^6/g respectively. The results with respect to TTA were also similar (Table 2). Raccach (1987) obtained 39% reduction in fermentation time of meat sausages by increasing the rate of inoculum from log 7.5 to log 8.8 cfu/g.

Increase in LAB counts was lesser as the rate of inoculum increased which has been observed earlier (Marcy *et al.*, 1985; Twiddy *et al.*, 1987). Higher LAB inoculum levels suppressed the growth of spoilage microflora and reduced TMA and TVB levels. Similar observation has been made by Adams *et al.* (1987).

The organoleptic analysis of sausages with different levels of LAB inocula showed no differences, but sausages with 10^8/g inoculum received slightly lower scores for taste and overall acceptability (Table 3), perhaps because of their lower pH. However, the panelists failed to recognise any off odours in sausages with 10^6/g and 10^7/g LAB inocula, where the TPC was 10^8/g and 10^7/g respectively. Although the TVB and TMA values for these samples were within the border limits, the TPC values certainly indicate that the samples were not fit for consumption, since 10^7/g of TPC has been considered by many workers as a limit for acceptability (Hobbs, 1953; Shewan, 1962 and Thatcher & Clark, 1968). The reason for not identifying the off flavours by the panelists appears to be the fairly high levels of spices used in these sausages which may have masked the off flavours. It has been opined by several workers that TVB and TMA-N also lose their usefulness as indicators of spoilage in fermented and heavily spiced sausages, fish and meat products like pickles and cured products (Pillai *et al.*, 1956; Lahiry *et al.*, 1961; Sen *et al.*, 1961, 1964 and Adebona, 1978). From the safety point of view, the inoculum rate of 10^8/g was finally selected for all further studies. This was also in conformity with the rate of inoculum used by other workers (Lindgren & Pleje, 1983; Raccach, 1986 and Twiddy *et al.*, 1987).

The results indicated that the rate of fermentation was not very much influenced by different sugar levels (Table 4). At all levels of sugar the pH of the sausages reached 5.1 (approximately) in 10 h of fermentation and slightly lower pH was observed in the final products at higher sugar levels. With respect to utilisation of sugar, during the fermentation period and smoking period (total 12 h), only 0.35% of sugar was utilised at 1% sugar level, 0.98% at 2% and 1.7% at 5% sugar level (Table 5). These results indicate that the percentage utilisation of sugar increased with

Table 2. *Changes in the pH, TTA*, LAB counts**, TPC**, TVB*** & TMA*** of semi dry sausages prepared with various levels of LAB inoculum.*

Level of LAB inoculum	10^6 cfu/g (0.02%)						10^7cfu/g (0.2%)					
	pH	TTA	LAB counts	TPC	TVB	TMA	pH	TTA	LAB counts	TPC	TVB	TMA
Immediately after stuffing and linking	6.6	0.22	$4.6x10^6$	$6.91x10^4$	8.50	0.85	6.6	0.23	$4.6x10^7$	$6.91x10^4$	8.41	0.86
Fermentation time to reach pH 5.1				22h						17h		
After fermentation (pH 5.1)	5.1	0.81	$9.8x10^7$	$8.2x10^7$	28.15	3.40	5.10	0.81	$8.5x10^8$	$1.14x10^7$	20.25	2.30
After 2h of smoking	4.95	0.875	$8.6x10^7$	$7.95x10^7$	31.25	4.7	4.95	0.875	$7.31x10^8$	$9.86x10^6$	24.50	3.20

Table 2. Continued...

Level of LAB inoculum	10^8cfu/g (2%)					
	pH	TTA	LAB counts	TPC	TVB	TMA
Immediately after stuffing and linking	6.6	0.21	$3.90x10^8$	$7.15x10^4$	8.45	0.83
Fermentation time to reach pH 5.1				10h		
After fermentation (pH 5.1)	5.15	0.81	$4.16x10^9$	$4.12x10^5$	12.45	1.65
After 2h of smoking	4.90	0.896	$2.93x10^9$	$3.8x10^5$	16.50	1.85

*TTA Values expressed as percent lactic acid
**LAB and TPC represent cfu g^{-1}
***TVB and TMA values expressed as mg N%

the increased sugar levels and this trend is reflected in the growth of LAB cultures and the spoilers (Table 5). It appears from the results that the excess sugar at higher levels has been utilised mostly for growth than for acid production, since no significant increase in acid production was noticed among different sugar levels. Adams *et al.* (1987) working on fermentation of minced whiting with two commercial lactic cultures, namely *Lactobacillus plantarum* and *Pediococcus pentosaceus* observed that the pH decreased faster with increase of glucose or sucrose content upto 5%, w/w, while Olsen (1985) reported that rate of fermentation depends upon the type and levels of carbohydrates (0.1 to 1.0%). However, acid production did not increase indefinitely with sugar content,

Table 3. *Organoleptic evaluation of semi-dry sausages prepared with various levels of LAB inoculum and sugar*

Attributes	Levels of LAB inoculum, cfu g^{-1}			Levels of sugar				
	10^6 (0.02%)	10^7 (0.2%)	10^8 (2%)	1%	2%	3%	4%	5%
Appearence	7.22	7.22	7.21	7.10	7.11	7.11	7.12	7.11
Colour	7.11	7.11	7.11	7.10	7.10	7.10	7.10	7.12
Taste	7.00	7.00	6.50	7.66	6.33	5.66	5.66	5.66
Texture	7.31	7.30	7.31	7.21	7.20	7.21	7.20	7.20
Odour	7.11	7.12	7.11	7.30	7.31	7.30	7.30	7.31
Overall acceptability	7.00	7.00	6.50	8.00	7.50	6.10	6.10	6.10

Figures represent mean panel scores for each attribute

Table 4. *Changes in the pH and TTA of semi-dry sausages prepared with various levels of sugar.*

Levels of sugar	1%		2%		3%		4%		5%	
	pH	TTA	pH	TTA	pH	TTA	pH	TTA	pH	TTA
Immediately after stuffing and linking	6.50	0.26	6.5	0.26	6.5	0.26	6.5	0.26	6.5	0.26
After 10 h of fermentation	5.10	0.81	5.15	0.81	5.10	0.81	5.05	0.83	5.05	0.83
After 2h of smoking	4.95	0.87	4.85	0.92	4.75	0.96	4.70	0.98	4.70	0.98

Note: TTA expressed as percent lactic acid

as the pH became limiting below a critical level. The pH of the sausage decreased rapidly with respect to time in the beginning till the pH of 5.1 is reached and thereafter the decrease was minimal. Comparing acid production at different levels of sugar, no significant difference was seen during the first 10 h of fermentation. After this, however, a slightly increased acid production was seen at higher levels of sugar compared to 1% sugar level which is probably due to the slightly increased growth of LAB culture as seen from the Table 5.

The lack of increased acid production with higher sugar level in the initial period was probably due to non-inclusion of sugar levels below 1%. As is evident from the results, 1% sugar level itself was more than sufficient to achieve the objective of this fermentation, namely fast growth acid production to a pH of 4.5 to 4.8 in a minimum time. Olsen (1985) using four different levels of glucose (0.1 to 1%), with *L. plantarum* and *P. pentosaceus* could achieve a pH of 5.0 only after 30 to 31h as a lower rate of inoculum was used.

Table 5. *Changes in the LAB counts, TPC and sugar content of semi dry sausages prepared with various levels of sugar*

Levels of sugar	1%			2%			3%		
	LAB counts	TPC	Sugar %	LAB counts	TPC	Sugar %	LAB counts	TPC	Sugar %
Immediately after stuffing and linking	4.0×10^{8}	6.00×10^{4}	1.05	3.80×10^{8}	6.50×10^{4}	2.10	3.60×10^{8}	6.80×10^{4}	3.01
After 10 h of fermentation	4.12×10^{9}	2.99×10^{5}	-	3.95×10^{9}	3.55×10^{5}	-	3.90×10^{9}	3.75×10^{5}	-
After 2 h of smoking	2.72×10^{9}	2.85×10^{5}	0.70	2.51×10^{9}	3.31×10^{5}	1.12	2.46×10^{9}	3.45×10^{5}	1.85

Table 5 Contd...

Levels of sugar	4%			5%		
	LAB counts	TPC	Sugar %	LAB counts	TPC	Sugar %
Immediately after stuffing and linking	3.25×10^{8}	6.90×10^{4}	4.02	3.10×10^{8}	7.10×10^{4}	5.01
After 10 h of fermentation	5.10×10^{9}	3.80×10^{5}	-	6.20×10^{9}	3.95×10^{5}	-
After 2 h of smoking	3.63×10^{9}	3.56×10^{5}	2.75	4.73×10^{9}	3.70×10^{5}	3.31

Note: LAB and TPC counts are in cfu g^{-1}

Among the sausages containing different sugar levels, those containing 1% sugar level scored better in organoleptic evaluation (Table 3). While the mean panel scores for appearance, colour, texture and odour remained almost the same at all sugar levels, scores for attributes like taste and overall acceptability varied with different sugar levels. The mean panel scores given for taste and overall acceptability decreased with the increased sugar levels and 1% sugar level received the maximum scores for these attributes. Keeping in view the residual sugars left in the sausages after fermentation and smoking and the mean panel scores obtained in the organoleptic evaluation, 1% sugar level was chosen as the optimum.

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