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Effect of Different Levels of Spice Mixture and Salt on the Preparation of Semi-dried Fish Sausages

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Fermented semi-dry fish sausages were prepared with different levels of spice mixture (1 to 3.0%) and salt (1.5 to 3.5%) and were subjected to chemical and organoleptic evaluation. Different levels of spice mixture did not have any significant effect on the rate of fermentation and 2.5% level of spice mixture was preferred by the panelists. Increased salt levels delayed fermentation rate and 3% salt level was preferred by the panelists.

For over 2000 years, lactic bacteria have been used to make food products varying in flavour, composition and consistency. The use of lactic acid starter cultures in food products are well known. The present work was taken up with an intention to develop a method for the preparation of fermented semi-dry fish sausages, since no attempts have so far been made to develop fermented fish sausages except for a lone report on the preparation of Krill sausages using lactic acid bacteria (Christians, 1980). A rapid lactic acid bacterial (LAB) growth in the initial phase of fermentation of the sausage is necessary so as to suppress the simultaneous growth of the spoilers and pathogens. The fermentation rate is influenced by several factors; spices and salt being two factors among these.

There are several reports on the stimulatory action of spices on the LAB growth (Sreenivasamurthy & Krishnamurthy, 1958, Kissinger & Zaika, 1978; Zaika & Kissinger, 1979a, b and Yoo *et al.*, 1978) and the active component responsible was identified to be manganese (Zaika & Kissinger, 1984; Raccach & Marshall, 1985); however, Twiddy *et al.* (1987) observed no stimulation by Mn^{+2}. Besides, some spices like oregano, sage, etc were found to have adverse effect on LAB growth (Zaika *et al.*, 1983).

Therefore, as a part of standardisation of semi-dry sausages, different levels of spice mixture and salt 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.

Six LAB cultures namely Pa_2 (*Pediococcus acidilactici*), Pc (*Pediococcus cerevisiae*), Lp_1 (*Lactobacillus plantarum*) and Lp_2 (*Lactobacillus plantarum*) and La (*Lactobacillus acidophilus*), obtained from National Chemical Laboratory, Poona, were inoculated into fish mince: sugar (3%) medium and into the sausage mix (Table 1), at the rate of 10^9 cells/g, incubated at room temperature and their growth ascertained by the measurement of pH of the medium.

The composition given for meat sausages by Kramlich *et al.* (1973) and Bawa & Delong (1985) was adopted here with slight modifications after replacing animal meat with fish meat (Table 1). The order of

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addition of ingredients was : Fish mince, sugar, spices, colour, fat, salt and young LAB culture suspension. Sausages were prepared by varying the levels of salt, spice mixture and the composition of spice mixture.

Five different levels of spice mixture namely 1.0, 1.5, 2.0, 2.5 and 3.0 percent of sausage with the remaining ingredients were (Table 1) were prepared by keeping the composition of spice mixture constant. Three spice compositions (Group A : pepper - 0.35%, chilli - 0.40%; Group B : pepper - 0.40%, chilli - 0.35% and Group C : pepper 0.55%, chilli - 0.20%) were tried at the standard spice mix level. For the purpose of standardisation of salt level, sausages were prepared with different levels of salt namely 1.5, 2.0, 2.5, 3.0 and 3.5 percent of sausage mix and the remaining ingredients as in Table 1.

After mixing for a brief period of 5-10 min in a stainless steel vessel using a ladle, the sausage mix was stuffed into natural casing (Madhwaraj *et al.*, 1980). The sausage were linked at 5-7.5 cm intervals, washed and hung in the smoking chamber at room temperatures (25 to 28°C) and allowed to ferment till a pH of 5.0 to 5.1 was reached. Later the sausages were smoked at 35 to 40°C for 2 h, i.e. till a pH of 4.6 to 4.75 was reached (Kramlich *et al.*, 1973; Pederson, 1979 and Christians, 1980), cooled to room temperature and stored in refrigerator (6 ± 2°C) until further use.

The pH and total titratable acidity, (AOAC, 1975) lactic acid bacterial counts (LAB counts) and total plate counts (TPC) (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.

Results and Discussion

All the six LAB cultures namely, Pa_2 (*P. acidilactici*), Pc (*P. cerevisiae*), Lp_1 (*L. plantarum*), Lp_2 (*L. plantarum*) and La (*L. acidophilus*), could grow in the fish meat medium with 3% sugar, as well as in the semi-dry sausage mix containing various ingredients (Table 2). Among the six LAB cultures, four cultures namely, Pa_2, Pc, Lp_1 and Lp_2 showed better fermentation characteristics. Pa_2 (*P. acidilactici*) was, however, selected for all further studies, since it was slightly better than the other three cultures in acid production. Flavour production among the cultures was not evaluated.

Different levels of spice mixture did not affect the rate of fermentation as reflected by

Table 1. *Recipe of semi-dry sausage mix used for ascertaining the growth of LAB*

Ingredients	%
Fish	79.50
Hydrogenated vegetable fat	4.00
Spice mixture*	1.00
Sugar	4.00
Salt	1.50
LAB culture suspension	10.00
*Spice mixture composition	
Black pepper	0.35
Chilli powder	0.40
Garlic	0.10
Ginger	0.10
Coriander	0.05

Table 2. *Ability of the LAB cultures to ferment fish meat containing 3% sucrose and semi-dry sausage mix*

Culture		pH of Fish meat + 3% sugar + LAB culture		pH of semi-dry sausage mix	
		Initial	After 24 h	Initial	after 12 h
(Pa2)	*Pediococcus acidilactici*	6.75	3.95	6.65	4.30
(Pc)	*Pediococcus cerevisiae*	6.75	4.00	6.65	4.20
(Lp1)	*Lactobacillus plantarum*	6.75	4.00	6.65	4.20
(Lp2)	*Lactobacillus plantarum*	6.75	4.05	6.65	4.20
(Lp)	*Lactobacillus plantarum*	6.75	4.45	6.65	5.65
(La)	*Lactobacillus acidophilus*	6.75	4.45	6.65	5.40

Table 3. *Changes in the pH, TTA*, LAB counts** and TPC** of semi-dry sausages prepared with various levels of spice mix*

Percentage of spice mixture	1.0				1.5				2.0			
Time	pH	TTA	LAB counts	TPC	pH	TTA	LAB counts	TPC	pH	TTA	LAB counts	TPC
Immediately after stuffing & linking	6.45	0.22	1.34×10^{9}	7.14×10^{4}	6.45	0.22	1.23×10^{9}	6.5×10^{4}	6.45	0.22	1.05×10^{9}	6.9×10^{4}
After 7 h	5.15	0.81	7.5×10^{9}	4.51×10^{5}	5.10	0.81	6.95×10^{9}	4.23×10^{5}	5.10	0.81	7.2×10^{9}	3.56×10^{5}
After 2 h smoking	4.65	1.02	6.10×10^{9}	4.28×10^{5}	4.65	1.02	5.55×10^{9}	4.03×10^{5}	4.65	1.02	5.70×10^{9}	3.34×10^{5}

Table 3 Contd...

Percentage of spice mixture	2.5				3.0			
Time	pH	TTA	LAB counts	TPC	pH	TTA	LAB counts	TPC
Immediately after stuffing & linking	6.5	0.22	1.33×10^{9}	7.10×10^{4}	6.45	0.22	1.21×10^{9}	7.20×10^{4}
After 7 h	5.10	0.81	7.6×10^{9}	3.93×10^{5}	5.10	0.81	7.42×10^{9}	4.00×10^{5}
After 2 h smoking	4.65	1.02	6.2×10^{9}	3.74×10^{5}	4.65	1.02	5.92×10^{9}	3.81×10^{5}

*Values expresed as percent lactic acid; ** cfu per gram

the pH and acid production (Table 3). There are several reports on the enhancement of acid production by LAB in fermented meat products and in liquid medium in the presence of spices (Kissinger & Zaika, 1978; Zaika & Kissinger, 1979a, b, 1984; Nes & Skjelkvale, 1982 and Raccach & Marshall, 1985). However, in the present study, the minimum level of the spice mixture used was 1.0% which is equivalent to the highest level of spice mixture used in the experiments by Nes & Skjelkvale (1982).

The different levels of spices mix also did not affect the growth of LAB during 7 h of fermentation (Table 3). Marcy *et al.*, (1985) working on fermented meat sausages observed no correlation between acid production and LAB growth, whereas Eskeland & Nordal (1980) observed correlation between acid production and LAB growth. However, in this study no correlation was found between acid production and growth. The different levels of spice mixture also had no significant effect on the growth of spoilers or TPC (Table 3).

Increasing levels of spice mixture, however, seemed to increase the acceptability (Table 4) as 2.5% level of spice mixture, received the highest scores for taste and overall acceptability. This indicated that the Indian palate prefers higher level of spice mixture than the Westerners, who prefer 1% spice level in their products.

Among the different combinations (groups A, B & C) of spices, group C got the highest panel scores for taste and overall acceptability followed by combinations B & A (Table 4).

Table 4. *Organoleptic evaluation of semi-dry sausages prepared with different levels of spice mix, composition of spice mix and levels of salt*

Variable	Spice mix level					Spice mix composition			Salt level				
						Group	Group	Group					
Percentage or groups	1.00	1.50	2.00	2.50	3.00	A	B	C	1.50	2.00	2.50	3.00	3.50
Attributes													
Appearance	7.15	7.13	7.14	7.15	7.15	7.20	7.20	7.20	7.22	7.22	7.21	7.23	7.21
Colour	7.21	7.21	7.22	7.22	7.20	7.10	7.15	7.10	7.11	7.12	7.11	7.11	7.11
Taste	5.10	5.30	7.30	8.00	7.00	5.50	6.50	7.50	4.51	5.33	6.33	7.25	6.83
Texture	7.30	7.29	7.31	7.30	7.29	7.20	7.20	7.20	7.31	7.30	7.31	7.30	7.31
Odour	7.11	7.11	7.12	7.13	7.14	7.30	7.30	7.30	7.11	7.11	7.12	7.11	7.10
Overall acceptability	6.10	6.20	7.50	8.00	7.10	5.5	6.10	7.00	4.11	4.10	6.50	7.20	6.50

Note: Figures represent mean panel scores for each attribute

Table 5. *Changes in the pH, total titrable acidity (TTA)*, lactic acid bacterial counts (LAB)** and total plate counts** of semi-dry sausages prepared with various levels of salt*

Percentage of salt	1.5				2.0				2.5			
	pH	TTA	LAB Counts	TPC	pH	TTA	LAB Counts	TPC	pH	TTA	LAB Counts	TPC
Immediately after stuffing & linking	6.50	0.22	$1.34x10^{9}$	$7.10x10^{4}$	6.45	0.22	$1.32x10^{9}$	$6.6x10^{4}$	6.45	0.22	$1.40x10^{9}$	$6.91x10^{4}$
After fermentation	5.1	0.81	$7.6x10^{9}$	$4.51x10^{5}$	5.20	0.78	$6.92x10^{9}$	$4.37x10^{5}$	5.10	0.81	$6.4x10^{9}$	$6.26x10^{5}$
After 2 h of smoking	4.6	1.02	$6.10x10^{9}$	$4.25x10^{5}$	4.60	1.02	$5.52x10^{9}$	$4.17x10^{5}$	4.75	0.96	$5.1x10^{9}$	$4.06x10^{5}$
Fermentation time to reach pH 5.1			7 h				7 h				8 h	

Table 5 Contd...

Percentage of salt	3.0				3.5			
	pH	TTA	LAB Counts	TPC	pH	TTA	LAB Counts	TPC
Immediately after stuffing & linking	6.50	0.22	$1.32x10^{9}$	$7.10x10^{4}$	6.50	0.22	$1.34x10^{9}$	$7.25x10^{4}$
After fermentation	5.15	0.81	$5.52x10^{9}$	$4.11x10^{5}$	5.10	0.81	$5.35x10^{9}$	$3.98x10^{5}$
After 2 h of smoking	4.75	0.96	$4.32x10^{9}$	$3.95x10^{5}$	4.75	0.96	$4.15x10^{9}$	$3.75x10^{5}$
Fermentation time to reach pH 5.1			9 h				10 h	

*Values expressed as percent lactic acid; **counts per gram;

However, it was observed that increasing spice level and varying spice composition alone were not crucial in taste acceptability, since the panelists suggested the reduction of sourness in the product. The sourness noticed in these trials may be due to the lower salt level (1.5%) chosen to avoid higher intake of dietary sodium in fermented sausages and the adverse effect of higher levels of salt on the LAB growth (Terrel *et*

al., 1983; Marcy *et al.*, 1985; Mendoza & Owens, 1986 and Adams *et al.*, 1987).

Consequent to the above observations, different levels of salt ranging from 1.5 to 3.5% were tried. The sausages prepared with 3% salt were preferred by the panelists with respect to taste and overall acceptability (Table 4). However, this increased level of salt necessitated the extension of fermentation time by 2 h to attain the desirable pH of 5.1 (Table 5).

Increasing salt levels had an adverse effect on the fermentation rate and the growth of the LAB (Table 5). While the LAB counts were lesser by 0.15 log cycles, the time required for reaching pH 5.1 increased by another 3 h, when salt level was increased from 1.5 to 3.5%. Marcy *et al.* (1985) working on the fermented meat sausages and Adam *et al.* (1987) on fermented fish mince - carbohydrate substrates, also observed that increasing the salt levels resulted in increased fermentation time. Increased salt levels, however, appeared to have no significant effect on the total plate counts (Table 5).

In the fermented meat sausages, the salt level generally used is in the range of 2.5 to 3.5% (Pederson, 1979) and in some Danish sausages it is as high as 7.5% (Olsen, 1985). There has been concern about the higher intake of dietary sodium which has been correlated to high blood pressure and related problems. Terrel *et al.* (1983) have tried to replace sodium with calcium and/or magnesium by 50%, but with adverse effect on flavour, water activity and survival of *Tricheana spiralis*. Marcy *et al.* (1985) suggested to reduce the salt level to 1.65% from 3.3%. However, from the acceptability point of view, one cannot afford to reduce the salt level to less than 3.0% in the fermented meat sausages.

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References

Adams, M.R., Cooke, R.D. & Twiddy, D.R. (1987) *Int. J. Food Sci. Technol.* **22**, 105

AOAC (1975) *Official Methods of Analysis*, 12th edn., Association of Official Analytical Chemists, Washington D.C., USA

Bawa, A.S. & Delong, R.O. (1985) *J. Food Sci. Technol.* (Mysore) **22**, 262

Christians, O. (1980) in *Advances in Fish Science and Technology* (Connell, J.J., Ed.), p.322, Fishing News (Books) Ltd. Farnham, England

Eskeland, B. & Nordal, J. (1980) *J. Food Sci.* **45**, 1153

Harrigan, W.F. & Mc Cance, M.E. (1976) in *Laboratory methods in Food and Dairy Microbiology*, Academic Press, London

Kissinger, J.C. & Zaika, L.L. (1978) *J. Food Prot.* **41**, 429

Kramlich, W.E., Pearson, A.M. & Tauber, F.E. (1973) *Processed Meats*, The AVI Publishing Co., Inc., West Port, Connecticut, USA

Madhwaraj, S., Nair, K.K.S., Nair, R.R., Kadkol, S.B. & Baliga, B.R. (1980) *J. Food Sci. Technol.* **17**, 273

Marcy, J.A., Kraft, A.A., Olson, D.G., Walker, H.W. and Hotchkiss, D.K. (1985) *J. Food Sci.* **50**, 316

Mendoza, L.S. & Owens, J.D. (1986) in *Proceedings of the First Asian Fisheries Forum* (Maclean, *et al.*, Ed.), p.443, Asian Fisheries Society, Manila, Phillippines

Nes, I.F. & Skjelkvale, R. (1982) *J. Food Sci.* **47**, 1618

Olsen, L.F. (1985) *J. Sci. Food Agri.* **36**, 1341

Pederson, C.S. (1979) *Microbiology of Food Fermentations*, 2nd edn., p.210, AVI Publishing Company, Inc. West Port CT, USA

Raccach, M. & Marshall, P.S. (1985) *J. Food Sci.* **50**, 665

Sreenivasamurthy, V. & Krishnamurthy, K. (1958) *Food Sci.* (Mysore), **8**, 24

Terrel, R.N., Ouintanilla, M., Vanderzant, C. & Gardner, F.A. (1983) *J. Food Sci.* **48**, 122

Twiddy, D.R., Cross, S.J. & Cooke, R.D. (1987) *Int. J. Food Sci. Technol.* **22**, 115

Yoo, J.Y., Min. B.Y. Suh, K.B. & Hah, D.M. (1978) *Korean J. Food Sci. Technol.* **10**, 124

Zaika, L.L. & Kissinger, J.C. (1979a) *J. Food Prot.* **42**, 572

Zaika, L.L. & Kissinger, J.C. (1979b) Paper 367 presented at the 39th Annual Meeting, Institute of Food Technologists, St. Louis, MO, June 10-13

Zaika, L.L. Kissinger, J.C. & Wasserman, A.E. (1983) *J. Food Sci.* **48**, 1455

Zaika, L.L. & Kissinger, J.C. (1984) *J. Food Sci.* **49**, 5