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RESEARCH ARTICLE

CHARACTERISTICS OF LACTIC ACID BACTERIA BEING PROPOSED AS STARTER CULTURES FOR EXTENDING THE SHELF LIFE OF A NIGERIAN GRILLED MEAT PRODUCT *TSIRE*

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ABSTRACT

This finding was aimed at investigating the characteristics of selected strains of lactic acid bacteria (LAB) being proposed for use as starter cultures for extending the shelf life of *tsire*, a Nigerian grilled meat product. The LAB strains used included *Lactobacillus plantarum*, *Streptococcus lactis*, *S. bovis*, *Pediococcus acidilactici* and *Pediococcus* sp., which were previously isolated from beef. The pH value of below 4.5 was recorded in growth medium of the LAB isolates. The highest concentration of lactic acid (2.9 mg/l) was recorded for *L. plantarum*, while *Pediococcus* sp. had the lowest (1.2). Acetic acid production was below 0.4 mg/l by all isolates. Proteolytic and lipolytic activities were not recorded by the strains, except *Pediococcus* sp. which recorded proteolytic activity of 0.03 unit/ml. Inclusion of spice combination consisting ground red pepper, tomato and onion in growth had a Mg^{2+} content of 17.1 ppm, and gave the highest support to the growth of LAB strains *L. plantarum* and *S. lactis*; values of 9.8 and 8.8 were obtained as log CFU/ml for the respective organisms. *Tsire* samples treated with cultures of *L. plantarum* and *S. lactis* and stored at 30ºC had lower thiobarbituric acid and free fatty acid values than uninoculated control counterparts. It was concluded that non proteolytic and lipolytic activities with reduction in acetic acid production as well as relatively high production of lactic acid would constitute significant characteristics of LAB strains to be adopted as starter cultures in extending the shelf life of a Nigerian grilled meat product *tsire*. Optimization of spice combinations in medium of the LAB cultures may also affect their growth and metabolic activities towards production of useful antimicrobial compounds, especially lactic acid.

INTRODUCTION

A known culture of microorganism used to inoculate foods in order to produce desired changes through fermentation is known as starter culture (Bohme *et al.*, 1996). Lactic acid bacteria (LAB) have a generally regarded as safe (GRAS) status and have been widely used as starters in the industrial preservation of meats (Simpson *et al*., 2002). The use of LAB as starter cultures in the manufacture of fermented meat products has received considerable interest and is very common in the meat industry (Schillinger and Lucke, 1989). Most used starter cultures are homofermentative LAB such as *Lactococcus*, *Pediococcus* and some species of *Lactobacillus* which produce lactic acid as the major carbohydrate metabolite giving the characteristic taste and flavour development as well as preservation of the fermented meat products (Bohme *et al.*, 1996; Zhao *et al*., 2013). The heterofermentative types of LAB such as *Leuconostoc* would produce other metabolites in addition to lactic acid and can lead to defect in the fermented meat sausage (Shay and Egan, 1991). The use of LAB as starter cultures has been found to reduce the fermentation time and improve safety of most fermented food products, especially in meat processing and preservation. *Tsire* is a grilled meat product that is commonly consumed as delicacies in Nigeria. Sellers of the product don't normally exhaust their sales on the day of production, and therefore some are left till the second day or beyond. This increases chances of microbial contamination of the product as a result of inadequate storage facilities; hence the use of biological agents for their preservation are required (Olaoye and Onilude, 2010). Many spices such as tomatoes, onions, garlic and pepper are usually used as additives during preparation of *tsire*. This research work thus aimed at investigating the effect of these spices on production of antimicrobial agents by selected strains of LAB in order to ascertain their suitability as starter cultures in enhancing shelf life of the product. The performance of some of the LAB strains in extending the shelf life of *tsire* was also evaluated during a seven day storage.

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MATERIALS AND METHODS

Sample collection

The spices and meat (beef) used for this research work were obtained from Bodija retail market in Ibadan metropolis, Oyo State, Nigeria. They were taken to the laboratory in sterile polyethylene bags for immediate use; meat was conveyed via ice crystals to prevent possible deterioration due to microbial activity.

Lactic acid bacteria used

The LAB isolates used in this study were *Lactobacillus plantarum, Streptococcus lactis, S. bovis, Pediococcus acidilactici* and *Pediococcus* sp., which have been isolated in a previous study (Olaoye and Onilude, 2009).

Growth of lactic acid bacteria

Broths of deMann Rogosa Sharpe (MRS) were used as growth medium for the LAB cultures. Sterile MRS broths were inoculated with respective cultures under aseptic conditions and then incubated at 30^0C for 18-24 h. The cultured broths were then subjected to centrifugation at 5000 × *g* for 15 min (Centrifuge Falcon 6/300 series, CFC Free, UK) to obtain cell free supernatant (CFS). The CFS of the LAB was then subjected to physiological and fermentative tests.

Determination of pH

This was carried out using the method described by Guererro *et al*. (1995). pH values were determined before and after incubation of growth medium.

Determination of lactic and acetic acids

The lactic and acetic acids were determined in CFS of each of the LAB cultures. The method used was as described by the AOAC (1990).

Determination of proteolytic activity

The method used for determination of proteolytic activity by the LAB strains was as described by Drici *et al*. (2010); extracellular proteolytic activity was evidenced by a transparent halo around bacterial colonies developing on milk agar because of hydrolysis of milk casein.

Determination of lipolytic activity

This was carried out using modified method of Yong and Wood (1977). The substrate used was prepared by mixing 20 ml glycerol tributyrate, 0.08 g sodium taurocholate, $0.2x10^{-2}$ g/ml $CaCl_2$, 2 g gum acacia (stabilizer) and 120 ml of 0.1 mol/l acetate buffer (pH 5.0). The mixture was blended into a fine emulsion using a warring blender. Thereafter, the culture filtrate (~0.5 ml) was added to 1 ml of the substrate and the mixture was shaken to mix before incubated at 40^oC for 1 h. The reaction was terminated by the addition of 8 ml ethanol (97% v/v). A volume of substrate (1 ml) without culture filtrate served as blank. The mixtures were titrated against 0.02 mole/l NaOH with phenolphthalein as indicator. The amount of alkali required to neutralize the liberated fatty acids was obtained from the difference in the titre values of the blank and reaction mixtures.

Determination of manganese and magnesium ions

These were determined by flame atomic absorption spectroscopy (Varian Model AA-1475) in duplicate samples, using the wet ashing method of AOAC (1990).

Performance of LAB cultures in different combinations of spices

The different spices normally used in *tsire* preparations were incorporated into the growth medium (MRS, pH 6.5) at 1% w/v to evaluate their effect on the different isolates; individual ground spices (roller mill produced), double combinations (1:1) and triple combinations (1:1:1) were tested. Each culture was inoculated into the spiced growth medium as 1 ml of a liquid suspension of the organism in sterile MRS broth. The mixture was shaken well and then incubated at 30 ± 3^0C for 24 h. Counts of LAB cultures were measured using the serial dilution technique and expressed as logarithm of colony forming unit per milliliter (log CFU/ml).

Performance of LAB cultures as starter cultures in extending the shelf life of *tsire*

Tsire samples were prepared and inoculated with *L. plantarum* and *S. lactis* as starter cultures as described by Onilude *et al.* (2002). Uninoculated samples served as control. The *tsire* samples were stored for seven days at 30^oC. During storage, samples were analysed for coliforms, *Staphylococcus*, *Micrococcus*, *Pseudomonas*, LAB, yeast and moulds, thiobarbituric acid (TBA) and free fatty acids (FFA) using the detailed methods of Onilude *et al.* (2002).

RESULTS

Table 1 shows the physiological and fermentative properties of the LAB isolates. All the organisms gave reduced pH values in growth medium, with *Pediococcus acidilactici*, *Streptococcus lactis* and *Lactobacillus plantarum* having values of 4.1, 4.3 and 4.0 respectively.

Table 1. Physiological and fermentative properties of the lactic acid bacteria

Isolates	pH	Lactic acid (mg/l)	Acetic acid (mg/l)	Proteolytic activity	Lipolytic activity
Pediococcus acidilactici	4.1	2.8	0.08	nd	nd
Pediococcus sp.	4.4	1.2	0.34	0.03	nd
Streptococcus lactis	4.3	2.6	0.02	nd	nd
Streptococcus bovis	4.5	1.5	0.38	nd	nd
Lactobacillus plantarum	4.0	2.9	0.04	nd	nd

nd = Not detected

The highest concentration of lactic acid (2.9 mg/l) was recorded for *L. plantarum*, and the lowest for *Pediococcus* sp. The highest acetic acid (0. 38 mg/l) was recorded for *S. bovis* while *S. lactis* had the lowest value of 0.02 mg/l. All the cultures did not display proteolytic or lipolytic activities, except *Pediococcus* sp. which recorded proteolytic activity of 0.03 unit/ml. Presented in Table 2 is the result of the effect of spices on the growth of the LAB cultures. Concentration of manganese and magnesium ions (Mn^{2+} and Mg^{2+}) in the different spices seem to have some effect on the growth of the LAB isolates. Of all the spices tested, the combination of ground red pepper, tomato and onion, having the highest Mg^{2+} content of 17.1 ppm, gave the highest support to the growth of cultures *L. plantarum* and *S. lactis*; growths of 9.8 and 8.8 log CFU/ml were recorded for the respective cultures. The growth of *P. acidilactici* was optimally supported by combination of ground red pepper and onion, having the highest log CFU of

Table 2. Effect of spices on the growth of lactic acid bacteria

Spices	Mineral content (ppm) Mn^{2+}	Mg^{2+}	*P. acidilactici*	*L. plantarum*	*S. lactis*	*S. bovis*
			log CFU/ml			
Ground red pepper	0.62	16.2	6.8	7.8	5.5	4.2
Black pepper	0.69	16.2	7.5	8.8	7.6	5.4
Onion	0.80	15.9	9.2	7.6	6.6	7.4
Tomato	0.56	16.3	8.8	9.6	7.2	3.8
Black pepper + Onion*	0.71	16.8	7.2	7.6	7.5	4.6
Ground red pepper + Onion*	0.68	16.9	9.7	8.2	8.4	5.1
Tomato + Onion*	0.67	16.2	6.4	8.0	7.2	5.2
Ground red pepper + Tomato*	0.70	16.8	8.3	9.6	8.5	4.6
Ground red pepper + Tomato +Onion**	0.69	17.1	6.2	9.8	8.8	5.2

Values are means of three replicates
*, 1:1 mixture of components: **, 1:1:1 mixture of components

Table 3. Effect of spices on pH and organic acid production by the lactic acid bacteria

Spices	Mineral content (ppm) Mn^{2+}	Mg^{2+}	*P. acidilactici* pH	Lactic acid (mg/ml)	Acetic acid	*L. plantarum* pH	Lactic acid	Acetic acid	*S. lactis* pH	Lactic acid	Acetic acid	*S. bovis* pH	Lactic acid	Acetic acid
Ground red pepper	0.62	18.2	4.1	5.3	0.18	3.9	7.3	0.13	4.7	6.1	0.17	5.0	4.6	0.12
Black pepper	0.69	16.2	4.4	4.5	0.20	4.3	6.0	0.17	4.5	5.5	0.13	5.4	4.2	0.16
Onion	0.80	15.9	4.6	3.7	0.18	4.1	6.4	0.18	4.4	5.1	0.15	5.5	4.0	0.13
Tomato	0.56	16.3	4.6	3.6	0.17	4.4	5.5	0.13	4.5	5.3	0.13	5.4	4.3	0.14
Black pepper + Onion*	0.71	16.8	4.7	3.8	0.18	4.4	5.3	0.11	4.3	5.7	0.15	5.4	4.4	0.16
Ground red pepper + Onion*	0.68	16.9	4.5	4.7	0.12	4.3	5.7	0.12	4.3	5.8	0.12	5.5	4.4	0.15
Tomato + Onion*	0.67	16.2	5.0	3.9	0.14	4.5	5.2	0.11	4.3	5.3	0.13	5.5	4.3	0.13
Ground red pepper + Tomato*	0.70	16.8	5.1	4.5	0.15	4.4	5.4	0.16	4.3	5.7	0.16	5.4	4.5	0.17
Ground red pepper + Tomato +Onion**	0.69	17.1	4.2	5.8	0.09	4.1	7.8	0.18	4.5	5.3	0.12	4.9	4.7	0.14

*, 1:1 mixture of components: **, 1:1:1 mixture of components

Table 4. Microbial load, thiobabituric acid (TBA) and free fatty acid (FFA) contents of *tsire* samples stored at 30°C for 7 days

Storage period (Days)	Sample	Coliforms	*Staphylococcus*	*Micrococcus*	*Pseudomonas*	LAB	Yeasts/moulds	TBA	FFA
		Log cfu/g							
1	T1	2.45	2.55	4.71	4.51	5.61	3.20	0.22	0.45
	T2	2.15	2.35	4.31	4.21	5.11	3.67	0.24	0.46
	T3	2.79	2.91	4.76	4.44	4.54	3.34	0.25	0.42
2	T1	4.32	4.87	6.71	6.32	7.81	4.01	0.25	0.47
	T2	4.32	4.87	6.71	6.32	7.81	4.01	0.27	0.50
	T3	5.67	6.81	8.32	7.98	6.98	6.71	0.36	0.62
3	T1	3.98	3.71	5.61	5.43	8.21	4.32	0.43	0.51
	T2	3.88	3.41	5.51	5.48	8.98	4.86	0.45	0.53
	T3	7.65	7.98	9.12	9.82	7.98	8.54	0.64	0.71
4	T1	4.48	4.31	4.24	6.13	10.30	5.09	0.45	0.52
	T2	4.68	4.54	4.63	6.23	10.12	5.21	0.48	0.57
	T3	9.65	9.98	9.12	9.99	6.98	8.77	0.68	0.73
5	T1	4.76	4.61	4.72	6.32	10.65	5.24	0.47	0.52
	T2	4.68	4.54	4.63	6.23	10.12	5.21	0.52	0.62
	T3	9.55	9.78	9.52	9.89	6.87	8.99	0.67	0.72
6	T1	4.68	4.51	4.34	6.43	10.43	5.19	0.47	0.53
	T2	5.10	4.98	4.97	6.88	10.12	5.86	0.56	0.66
	T3	9.88	9.97	9.89	10.21	6.78	8.98	0.70	0.81
7	T1	4.32	4.68	4.56	6.23	11.10	3.20	0.42	0.49
	T2	5.12	4.97	4.99	7.23	11.40	4.54	0.49	0.65
	T3	10.01	9.98	10.23	10.43	7.01	9.32	0.68	0.79

T 1, *tsire* samples treated with *Lactobacillus plantarum*; T2, *tsire* samples treated with *Streptococcus lactis*; T3, Uninoculated *tsire* samples; TBA, thiobarbituric acid (mg malonaldehyde/kg); FFA, free fatty acid (KOH/g lipid)

9.7. Moreover, the highest log CFU (7.4) for *S. bovis* was obtained by the use of onion alone. The concentration of manganese ion (0.80 ppm) in onion was higher than other spices. Table 3 shows the effect of spices on pH and organic acid production by the LAB isolates. Lactic acid production by *L. plantarum* was higher than other isolates, having the highest concentration of 7.8 while the lowest value of 4.0 was obtained for *S. bovis.* The microbial load, TBA and FFA contents of *tsire* samples during storage are shown in Table 4. Coliforms, *Staphylococcus* and *Pseudomonas* were generally lower in *tsire* samples treated with *L. plantarum* and *S. bovis* when compared to the uninoculated control samples during the storage period. Expectedly, LAB counts increased in the *tsire* samples with period of storage, with the LAB inoculated samples recording higher counts over their uninoculated counterparts. Reduced TBA and FFA values were observed in LAB treated samples compared to control throughout the period of storage. It is interesting to note that within the first four days of storage, TBA values were below 0.5 in *tsire* samples treated with *L. plantarum* and *S. lactis* while values were higher than 0.5 in uninoculated control samples.

DISCUSSION

It was observed from the results of the physiological and fermentative tests that *L. plantarum* had the highest lactic acid production. This could be due to the more aciduric activity of this organism compared to other LAB isolates that were tested (Stiles, 1991). The result confirms the research finding of Nordal and Slinde (1980) who reported higher lactic acid production by *L. plantarum* ATCC 8014 than other strains of LAB that were evaluated. In terms of lactic acid production, values observed for *P. acidilactici* and *Streptococcus lactis* were similar to that of *L. plantarum*, but were however lower. Hence *L. plantarum, S lactis* and *P. acidilactici* could make good candidates of starter cultures for food biopreservation, especially in the preservation of *tsire*. The low concentrations of acetic acid recorded in this study for the LAB cultures could be due to their homofermentative nature (Nordal and Slinde, 1980). The use of homofermentative LAB has been reported to be desirable because of their ability to produce acetic acid in low or negligible concentrations; acetic acid may impart unpleasant taste on food products when compared to lactic acid (Papa *et al.*, 1995). Hence low values of acetic acid recorded for the cultures in this study could be desirable in processing and preservation of the Nigerian grilled meat product, *tsire*.

Proteolytic and lipolytic activities were not detected in the LAB cultures except *Pediococcus* sp. The non detection in *P. acidilactici*, *L. plantarum*, *S. lactis* and *S. bovis* is supported by Leroy *et al.* (2006) and Ammor and Mayo (2007) who observed that LAB generally do not possess strong proteolytic or lipolytic properties. This may also contribute positively to the choice of the LAB strains as starter cultures for extending shelf life of the meat product, as off flavours have been noted to be associated with proteolytic and lipolytic activities (Nordal and Slinde, 1980; Axelsson, 1998). Detection of manganese and magnesium ions (Mn^{2+}, Mg^{2+}) in the spice combinations in this study is in agreement with the findings of Coventry and Hickey (1993). The effect of magnesium ions on production of lactic acid by the LAB cultures also agree with reports of the research workers. Other research workers have noted that magnesium ions may have stimulatory effect on lactic acid production by LAB (Shay and Egan, 1991; Hugas *et al.*, 2002). Combination of spices seems to have effect on concentrations of Mn^{2+} and Mg^{2+}, although with variations. Concentration of lactic acid production was higher for *L. plantarun*, *S. lactis* and *P. acidilactici* than *S. bovis*; this may give the three LAB strains an added advantage over the latter because lactic acid plays very significant role in the display of antimicrobial activities against spoilage and pathogenic organism by producer organisms in meat preservation (Olaoye and Onilude, 2010).

A similar trend was noted in the log CFU of the organisms when spices were used in combination or individually. The low values of acetic acid recorded for the LAB cultures were expected because of their homofermentative nature; hence are and are expected to produce lactic acid as the main metabolite (Hassan and Frank, 2001). The decrease in pH values in growth medium of the cultures could obviously be attributed to their lactic acid production (Olaoye *et al.*, 2011). Inoculation of *tsire* samples with cultures of *L. plantarum* and *S. lactis* resulted in reduction of TBA values when compared to uninoculated control counterparts, suggesting that the LAB cultures could help prevent lipid oxidation in the grilled meat product (Sallama and Samejima, 2004). This implies that spoilage due to rancidity may be delayed or prevented in the meat product. TBA values of higher than 0.5 has been noted to trigger rancidity in meat during storage (Tsaknis *et al.*, 1999; Sallama and Samejima, 2004).

Conclusion

From the results obtained in this finding, combination of spices ground red pepper, tomato and onions provided an optimal condition for production of lactic acid by the LAB isolates, especially *L. plantarum* and *S. lactis.* When employing any of these isolates as candidates of starter cultures for preservation of *tsire*, the spice combination should be taken into serious consideration so as to provide enhanced production of lactic acid towards ensuring increased shelf life of the product. There was reduction in TBA values in *tsire* samples inoculated with *L. plantarum* and *S. lactis* during storage, and this may reduce rancidity development in the product. Hence, the LAB cultures could be employed in practical preservation studies for extending the shelf life of the Nigerian grilled meat product *tsire*. However, further investigation on the safety of the proposed LAB cultures is suggested, even though LAB have GRAS status.

REFERENCES

Ammor, M.S., and B. Mayo. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. *Meat Science*, 2007, 76: 138–146

AOAC. Official Methods of Analysis, 15th edn. Washington, DC: Association of Official Analytical Chemists. 1990, ISBN 0-93 558 442-0

Axelsson, L. Lactic acid bacteria; classification and physiology. In: Microbiology and Functional Aspects of Lactic Acid Bacteria (eds S. Salminen and A. Vonwright), 2nd edn, Marcel Dekker, Inc. New York. 1998, 1-72

Bohme, H.M., Mellet, F.D., Dicks, L.M.T. and Basson, D.S. Production of Salami from ostrich meat with strains of *Lactobacillus sake*, *L. curvatus* and *Micrococcus* sp. *Meat Science*, 1996, 44: 1973-1979

Coventry, M.J. and Hickey, M.W. Effects of spices and manganese on meat start culture activity. *Meat Science*, 1993, 33: 391-399

Drici, H., Gilbert, C., Kihal, M. and Atlan, D. Atypical citrate-fermenting *Lactococcus lactis* strains isolated from dromedary's milk. *Journal of Applied Microbiology*, 2010, 108: 647–657

Guererro, I, Mendiolea, R., Ponce, E. and Prado, A. Inoculation of lactic acid bacteria (LAB) on meat surfaces as a means of decontamination in semitropical conditions. *Meat Science*, 1995, 40: 397-411

Hassan, A.N. and Frank, J.F. Starter cultures and their use. In; Applied Dairy Microbiology (eds. E. H. Marth and J.L. Steele), 2nd edn. Marcel Dekker, Inc. New York. 2001, pp 151 -206

Hugas, M., Garriga, M., Pascual, M., Aymerich, M.T. and Monfort, J.M. Enhancement of sakacin K activity against *Listeria monocytogenes* in fermented sausages with pepper or manganese as ingredients. *Food Microbiology*, 2002, 19: 519-528

Leroy, F., Verluyten, J. and De Vuyst, L. Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology*, 2006, 106: 270-285

Nordal, J. and Slinde, E. Characterization of some lactic acid bacteria (LAB) used as starter cultures in dry sausage production. *Journal of Applied and Environmental Microbiology*, 1980, 40: 472-475

Olaoye, O.A. and Onilude, A.A. Isolation and Biochemical Profiles of Numerous Strains of Lactic Acid Producing Bacteria from Various Parts of a Domestic West African Goat (*Capra hircus*). *Australian Journal of Basic and Applied Sciences,* 2009, 3: 460-466

Olaoye, O.A. and Onilude, A.A. Investigation on the potential use of biological agents in the extension of fresh beef in Nigeria. *World Journal of Microbiology and Biotechnology*, 2010, 26: 1445–1454

Olaoye, O.A., Onilude, A.A. and Idowu, O.A. Microbiological profile of goat meat inoculated with lactic acid bacteria cultures and stored at 30°C for 7 days. *Food and Bioprocess Technology*, 2011, 4: 312-319

Onilude, A.A., Sanni, A.I. Olaoye, O.A. and Ogunbanwo, S.T. Influence of lactic cultures on the quality attributes of *tsire*, a West African stick meat. *World Journal of Microbiology and Biotechnology,* 2002, 18: 615-619

Papa, F., Zambonelli, C. and Grazia, L. Production of Milano style Salami of good quality and safety. *Journal of Food Microbiology*, 1995, 12: 912-915

Sallama, K.I. and Samejima, K. Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. *Lebenson Wiss Technology*, 2004, 37: 865-871

Schillinger, V. and Lucke, F. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Journal of Applied and Environmental Microbiology*, 1989, 55: 1901-1906

Shay, B.J. and Egan, A.F. Meat starter cultures and the manufacture of meat products. In; *Enzyclopedia of Food Science and Technology*, 1st edn, John willey and sons inc., Canada, 1991, 3: 1735-1744

Simpson, P.J., Stanton, C., Fitzgerald, G.F. and Ross, R.P. Genomic diversity within the genus *Pediococcus* as revealed by randomly amplified polymorphic DNA PCR and pulsed-field gel electrophoresis. *Applied Environmental Microbiology*, 2002, 68: 765-771

Stiles, M.F. Meat Microbiology. In: *Encyclopedia of Food Sci. and Technol*. 1st edn, John willey and sons Inc., Canada. 1991, 3: 1691-1695

Tsaknis, J., Lalas, S. and Evmorfopoulos, E. Determination of malondialdehyde in traditional fish products by HPLC. *Analyst*, 1999, 124: 843-845

Yong, F.M. and Wood, B.J.B. Microbiology and Biochemistry of soysauce fermentation. *Advances in Applied Microbiology*, 1977, 17: 157-194

Zhao, J., Xu, L., Wang, Y., Zhao, X., Wang, J., Garza, E., Manow, R. and Zhou, S. Homofermentative production of optically pure L-lactic acid from xylose by genetically engineered *Escherichia coli* B. *Cell Factories*, 2013, 12: 57-60

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