

Evaluation of the Lipolytic and Antioxidant Activities of Different Strains of Coagulase-negative *Staphylococci* in Fermented Sausages

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Introduction

- > Recent studies have indicated that coagulase negative staphylococci (CNS) are not only predominant but also exhibit greater biodiversity compared to lactic acid bacteria in fermented sausages.
- \succ Moderate lipid oxidation can produce appropriate levels of small aldehydes, ketones, and carboxylic acids, thereby enhancing the flavor of meat products. Conversely, excessive lipid oxidation results in the formation of large quantities of volatile compounds, associated with off-flavors and undesirable taste.
- > Currently, there is limited knowledge regarding the precise enzyme activities associated with lipid hydrolysis and oxidation of CNS strains and the effects of them on sausage fermentation.

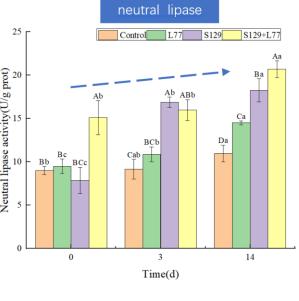
Objectives

In our previous study, we identified some CNS strains with remarkable lipolytic activity on tributyrin-containing agar and CAT activity in gas release tests, from Chinese spontaneously fermented meat products. In this study, we would like to evaluate the lipid hydrolase and antioxidant enzyme activities of six CNS strains in a meat simulation medium firstly, then examine the effects of the selected strain on lipid hydrolysis and oxidation in

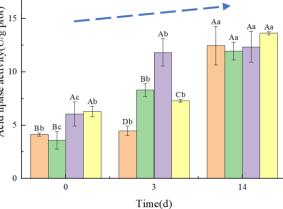


Lipid hydrolysis

					dig	lyceride: µmol/g,	others: g/100 g
Indexes	Time (d)	Control	L77	S129	S129+L77	Pooled SEM	P _{T×G}
	0	46.50 ^c	43.52 ^c	45.50 ^c	45.19 ^c	2.44	0.047
neutral lipids	3	65.53ª	61.29ª	64.53ª	63.11ª	2.93	
	14	60.36 ^b	59.50 ^b	62.19 ^b	59.51 ^b	2.19	
	0	9.36 ^{Ca}	9.50 ^{Ba}	10.50 ^{Aa}	9.33 ^{Da}	1.53	
phospholipid	3	4.25 ^{Cb}	4.50 ^{Bb}	3.51 ^{Dc}	6.53 ^{Ab}	1.02	0.058
	14	3.98 ^{Cc}	4.16 ^{Bc}	4.29 ^{Ab}	3.50 ^{Dc}	1.17	
	0	170.42 ^{Aa}	157.66 ^{Ba}	170.84 ^{Aa}	163.74 ^{ABa}	3.08	0.012
diglyceride	3	151.96 ^{Bb}	166.94 ^{Aa}	166.02 ^{Aa}	148.11 ^{Bb}	2.34	
	14	149.29 ^{BCb}	157.01 ^{ABa}	163.95 ^{Aa}	146.28 ^{Bb}	3.89	
	0	0.75 ^c	0.72 ^c	0.74 ^c	0.77 ^c	0.11	<0.001
monoglyceride	3	0.91 ^{Cb}	1.33 ^{Bb}	1.49 ^{Ab}	1.51 ^{Ab}	0.26	
	14	2.58 ^c	2.67 ^{Ca}	4.29 ^{Aa}	3.65 ^{Ba}	0.12	
	0	2.88 ^{ABc}	1.38 ^{Cc}	2.53 ^{Bc}	2.99 ^{Ac}	0.22	<0.001
free glycerol	3	2.90 ^{Cb}	2.49 ^{Db}	3.87 ^{Bb}	4.35 ^{Ab}	0.19	
	14	3.52 ^{Da}	5.04 ^{Ba}	4.77 ^{Ca}	5.87 ^{Aa}	0.08	
total fatty acids	0	6.63 ^{Cb}	9.20 ^{Ac}	9.39 ^{Ac}	8.57 ^{Bc}	0.89	<0.001
	3	19.03 ^{Ba}	22.57 ^{Ab}	13.11 ^{Db}	17.47 ^{Cb}	1.25	
	14	20.30 ^{Da}	24.47 ^{Ca}	33.68 ^{Aa}	30.72 ^{Ba}	1.52	



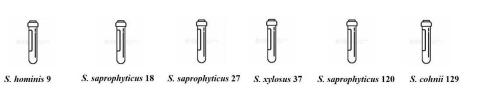


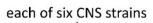


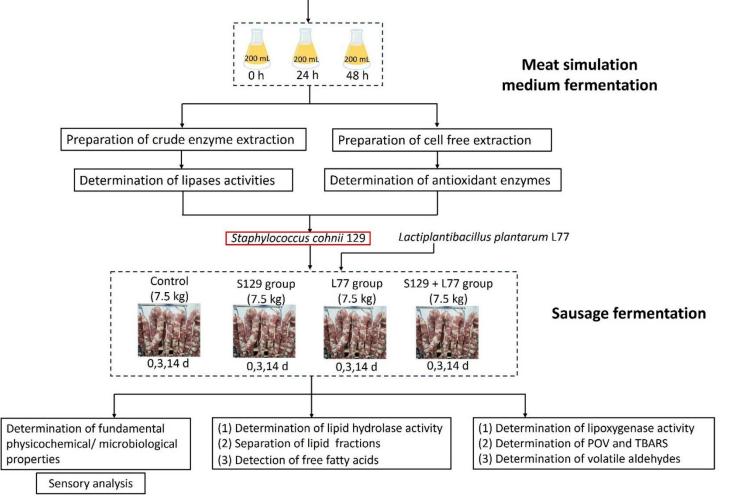
Total FFA: g/100g, others: mg/100 g

Indexes	Time(d)	Control	L77	S129	S129+L77	Pooled SEM	₽ _{T×G}
SFA subtotal [10]	0	3165.62 ^{Dc}	4228.00 ^{Bc}	3487.92 ^{Cc}	4359.86 ^{Ac}	23.87	<0.001
	3	8585.43 ^{Bb}	9488.47 ^{Ab}	5921.33 ^{Db}	7663.13 ^{Cb}	63.86	
	14	8890.00 ^{Da}	10645.08 ^{Ca}	13418.40 ^{Aa}	12953.87 ^{Ba}	61.96	
MUFA subtotal [9]	0	3568.72 ^{ABC}	3568.70 ^{Ac}	3487.86 ^{Bc}	3051.23 ^{Bc}	20.75	<0.001
	3	7482.87 ^{Bb}	9469.00 ^{Ab}	5219.84 ^{Db}	7035.41 ^{Cb}	73.24	
	14	7869 54 ^{Da}	9612 06 ^{Ca}	13765 43 ^{Aa}	12679 11 ^{Ba}	146.26	

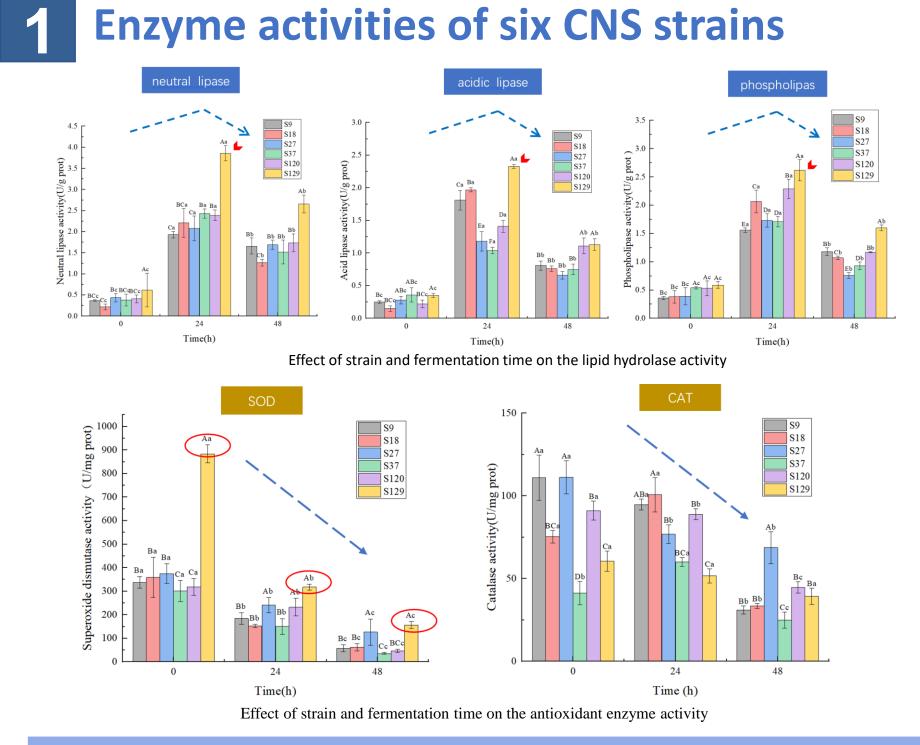
Experimental design



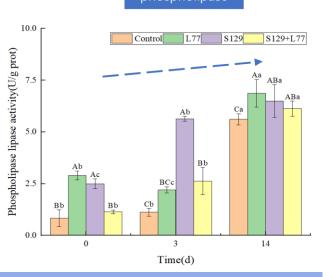




Results



0	1004.93 ^{Bc}	1407.36 ^{Ac}	1413.72 ^{Ac}	1161.23 ^{Bc}	23.16	
3	2961.22 ^{Bb}	3612.67 ^{Ab}	1967.35 ^{Db}	2769.13 ^{Cb}	27.21	<0.001
14	3541.91 ^{Da}	4208.86 ^{Ca}	6491.34 ^{Aa}	5087.30 ^{Ba}	60.88	
0	6.63 ^{Cb}	9.20 ^{Ac}	9.39 ^{Ac}	8.57 ^{Bc}	0.89	
3	19.03 ^{Ba}	22.57 ^{Ab}	13.11 ^{Db}	17.47 ^{Cb}	1.25	<0.001
14	20.30 ^{Da}	24.47 ^{Ca}	<u>33.68</u> ^{Aa}	30.72 ^{Ba}	1.52	
0	0.317	0.333	0.405	0.266		
3	0.345	0.381	0.332	0.361		
14	0.398	0.395	0.484	0.393		
	3 14 0 3 14 0 3	3 2961.22 ^{Bb} 14 3541.91 ^{Da} 0 6.63 ^{Cb} 3 19.03 ^{Ba} 14 20.30 ^{Da} 0 0.317 3 0.345	3 2961.22 ^{Bb} 3612.67 ^{Ab} 14 3541.91 ^{Da} 4208.86 ^{Ca} 0 6.63 ^{Cb} 9.20 ^{Ac} 3 19.03 ^{Ba} 22.57 ^{Ab} 14 20.30 ^{Da} 24.47 ^{Ca} 0 0.317 0.333 3 0.345 0.381	3 2961.22 ^{Bb} 3612.67 ^{Ab} 1967.35 ^{Db} 14 3541.91 ^{Da} 4208.86 ^{Ca} 6491.34 ^{Aa} 0 6.63 ^{Cb} 9.20 ^{Ac} 9.39 ^{Ac} 3 19.03 ^{Ba} 22.57 ^{Ab} 13.11 ^{Db} 14 20.30 ^{Da} 24.47 ^{Ca} 33.68 ^{Aa} 0 0.317 0.333 0.405 3 0.345 0.381 0.332	3 2961.22 ^{Bb} 3612.67 ^{Ab} 1967.35 ^{Db} 2769.13 ^{Cb} 14 3541.91 ^{Da} 4208.86 ^{Ca} 6491.34 ^{Aa} 5087.30 ^{Ba} 0 6.63 ^{Cb} 9.20 ^{Ac} 9.39 ^{Ac} 8.57 ^{Bc} 3 19.03 ^{Ba} 22.57 ^{Ab} 13.11 ^{Db} 17.47 ^{Cb} 14 20.30 ^{Da} 24.47 ^{Ca} 33.68 ^{Aa} 30.72 ^{Ba} 0 0.317 0.333 0.405 0.266 3 0.345 0.381 0.332 0.361	3 2961.22 ^{Bb} 3612.67 ^{Ab} 1967.35 ^{Db} 2769.13 ^{Cb} 27.21 14 3541.91 ^{Da} 4208.86 ^{Ca} 6491.34 ^{Aa} 5087.30 ^{Ba} 60.88 0 6.63 ^{Cb} 9.20 ^{Ac} 9.39 ^{Ac} 8.57 ^{Bc} 0.89 3 19.03 ^{Ba} 22.57 ^{Ab} 13.11 ^{Db} 17.47 ^{Cb} 1.25 14 20.30 ^{Da} 24.47 ^{Ca} 33.68 ^{Aa} 30.72 ^{Ba} 1.52 0 0.317 0.333 0.405 0.266



Lipase activities and the concentrations of lipid hydrolysis products, including monoglyceride, free glycerol, and free fatty acids, were higher in both S129 and S129+L77 than in the control.

Lipid oxidation

Indexes	Time(d)	Control	L77	S129	S129+L77	Pooled SEM	P _{T×G}
LOX (U/g protein)	0	775.13 ^{Aa}	501.86 ^{BCa}	479.89 ^{Ca}	573.63 ^{Ba}	52.74	<0.001
	3	502.80 ^{Ab}	444.03 ^{Ba}	388.69 ^{Ca}	398.64 ^{Cb}	43.90	
	14	375.17 ^{Ab}	95.43 ^{Cb}	253.98 ^{Bb}	302.67 ^{ABb}	40.32	
POV (mg/100 g)	0	90.15 ^{Ab}	60.76 ^{Ca}	70.07 ^{Ba}	90.67 ^{Aa}	10.74	<0.001
	3	100.22 ^{Ab}	30.36 ^{Cb}	40.11 ^{Cb}	60.38 ^{Bb}	8.91	
	14	130.68 ^{Aa}	60.14 ^{Ca}	70.65 ^{Ba}	70.69 ^{Bb}	5.67	
TBARS (mg/kg)	0	0.10 ^{Bc}	0.10 ^{Bc}	0.10 ^{Bb}	0.13 ^{Ab}	0.01	<0.001
	3	0.19 ^{Ab} 0.30	0.15 ^{Bb}	0.12 ^{Bb} 0.2	3 0.13 ^{Bb} 0.1	.7 0.01	
	14	0.40 ^{Aa} 🔔	0.33 ^{Ba}	0.33 ^{Ba} 1	0.30 ^{Ca} 1	0.02	

The suppression of lipoxygenase activity, TBARS hexanal, and saturated aldehyde production was more pronounced in S129 and S129+L77 compared to the control

unit: µg/kg

Indexes	Time(d)	Control	L77	S129	S129+L77	Pooled SEM	₽ _{T×G}
	0	54.98 ^{Aa}	44.90 ^{Ba}	45.81 ^{Ba}	35.34 ^{Cb}	4.41	<0.001
Hexanal	3	24.49 ^{Bc}	15.24 ^{Cc}	39.22 ^{Ab} 21.5	52 29.21 ^{Ac}	2.72	
	14	42.91 ^{Ab}	35.96 ^{Bb}	24.29 ^{cc} 🚅	37.96 ^{Ba}	1.79	
	0	238.19 ^{Bb}	167.89 ^{Cc}	156.35 ^{Cc}	325.99 ^{Ab}	44.28	<0.001
Nonanal	3	275.01 ^{Ba}	226.71 ^{Ca}	298.44 ^{Ba}	410.36 ^{Aa}	13.88	
	14	180.89 ^{Cc}	176.15 ^{Bb}	183.93 ^{Bb}	311.40 ^{Ac}	6.64	
Saturated	0	389.26 ^{Aa}	226.23 ^{Cb}	315.46 ^{Bb}	389.15 ^{Ab}	14.14	0.004
aldehyde	3	333.92 ^{Cb}	260.08 ^{Da}	451.07 ^{Ba}	476.37 ^{Aa}	18.11	
subtotal [6]	14	254.43 ^{Bc}	222.04 ^{Cc}	242. 208.22 ^{Dc}	400.12 ^{Ac}	9.46	
	0	64.21 ^{Aa}	37.21 ^{Da}	58.89 ^{Ba}	37.45 ^{Ca}	4.54	0.071
Trans-2-enal subtotal [2]	3	-	-	21.23 ^{Ab}	14.82 ^{Bb}	1.11	
	14	-	-	-	-		
	0	412.33 ^{Aa}	266.88 ^{Ca}	342.47 ^{Bb}	415.23 ^{Ab}	29.73	
Total [8]	3	333.75 ^{Cb}	260.08 ^{Db}	467.30 ^{Ba}	490.07 ^{Ba}	32.78	0.139
	14	259.56 ^{Bc}	225.15 ^{Cc}	217.02 ^{Cc}	402.71 ^{Ac}	46.33	

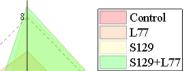
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The highest scores for overall

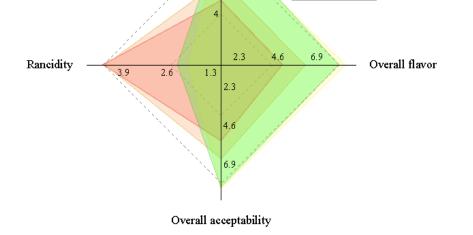
flavor and acceptability, and

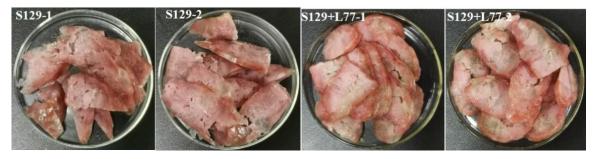
the lowest score for rancid

notes, were reported for S129









Conclusion

Our findings suggest that *Staphylococcus cohnii* 129, a relatively uncommon CNS strain, has the potential to serve as a functional starter culture, promoting lipid hydrolysis and mitigating lipid peroxidation, ultimately enhancing the overall quality of fermented sausages.

Above all, S. cohnii 129 exhibited remarkable lipid hydrolase activity and SOD activity. Hence, S. cohnii 129 was selected as the optimal strain to ferment sausage either alone (S129) or in combination with Lactiplantibacillus plantarum 77 (S129+L77), with a blank group as the control.

References

Acknowledgements

- 1. Woldmaiam, K. Y., Wang, Z.K., Cai, M., Li, M., Jiang, W.X., Hu, Z.C., Li, J.J., Tang, W.S., Jiao, Y.S., Liu, Y.L., Zheng, Q.K., & Wang, J. (2024). Lipid hydrolysis, oxidation, and fatty acid formation pathway mapping of synergistically fermented sausage and characterization of lipid mediating genes. Journal of Agricultural and Food Chemistry, 72: 17536-17548. 2. Vestergaard, C. S., Schivazappa, C., & Virgili, R. (2000). Lipolysis in dry-cured ham maturation. *Meat Science*, 55(1), 1–5. 3. Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive review on lipid oxidation in meat and meat products. Antioxidants (Basel, Switzerland), 8(10), E429.
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