EFFECT OF *STAPHYLOCOCCI* AND LACTIC ACID BACTERIA ON THE OXIDATION OF UNSATURATED FREE FATTY ACIDS

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KEYWORDS : lipid oxidation, starter culture, lactic acid bacteria, *Staphylococci*.

BACKGROUND

In sausage, starter cultures are widely used in order to shorten the ripening period, ensure colour development, enhance the flavour and improve product safety. Lactic acid bacteria (*Lactobacillus-Pediococcus*) produce lactic acid from carbohydrate fermentation which contribute to the acidification of the product (Hammes *et al.*, 1990 , Montel *et al.*, 1993). *Micrococcaceae* (*Staphylococcus-Micrococcus*) ensure colour development by nitrate reductase activity. They also contribute to the development of dry sausage aroma by influencing the composition of volatile compounds in the products (Berdagué *et al.*, 1993 ; Montel *et al.*, 1996 ; Stahnke 1994). Among these volatiles, they modify the level of compounds originating from lipids oxidation. Some of them such as ketones and secondary alcohols are involved in dry cured aroma, others like alkanes, alkenes and high level of aldehydes are undesirable compounds involved in rancidity. But the literature in the effect of bacteria on the oxidation of lipids and free fatty acids in meat fermentation is not well documented.

OBJECTIVES

The objective of this work was to characterise the role of different *Staphylococci* and lactic acid bacteria on the oxidation of unsaturated fatty acids with the aim to select flavour-improving starter cultures for cured meat products.

METHODS

Bacterial strains : The following *Staphylococci* : *Staphylococcus carnosus* (833, 836), *Staphylococcus xylosus* (831, 873, 16), *Staphylococcus warneri* (863), *Staphylococcus saprophyticus* (852) and Lactic Acid Bacteria : *Carnobacterium divergens* (210), *Carnobacterium piscicola* (545), *Lactobacillus sake* (206), *Lactobacillus plantarum* (Lpl), *Lactobacillus curvatus* (411), *Pediococcus pentosaceus* (716) were studied.

Media : Oxidation was studied in MC media (meat extract 10g/l ; yeast extract 5g/l ; Na_2HPO_4 2g/l ; NaCl 5g/l ; glucose 1g/l ; agar 3g/l) at pH 6.0). After sterilisation, either melted pork fat, oleic acid, linoleic acid, or linolenic acid was added to a final concentration of 0.5g/l. For the staphylococci all these substrates were studied, whereas for lactic acid bacteria only linoleic acid was studied in the two media : MC and MCMn, which is MC supplemented with manganese MCMn (0.05 g/l). The media were emulsified with an Ultra Turrax T25 homogenizer at 100000 rpm for 1 min. The media (4 ml) were distributed in small flasks and then inoculated.

The strains were inoculated at approximately 10^6 cells/ml. All samples, inoculated and controls, were incubated at 25°C and samples were analyzed after 0, 2, 5, 8, 15 and 20 days for staphylococci and 0, 2, 5, 8 and 15 days for LAB.

Analysis : Oxidation was evaluated by Thiobarbituric Acid reaction (TBA-RS) (Lynch and Frei, 1993). Results are expressed in µmoles of malonaldehyde/g of lipids.

RESULTS AND DISCUSSION

Oxidation of the substrates in the steril control : It was clear that oxidation depended mainly on the type of substrates. In fact pork fat and mono-unsaturated (C18:1) were poorly oxidized during all the incubation period (Table 1). Only tri-unsaturated (C18:3) and di-unsaturated (C18:2) fatty acids were sensitive to oxidation (Table 1). The oxidation of the two poly-unsaturated fatty acids started at the beginning of the incubation and reached a maximal value after 5 days of incubation in the control steril media MC (Table 1). The decrease of the TBA-RS values after 5 days was certainely due to apparition of volatiles compounds not assayed by this method. However, the presence of manganese in the media influenced greatly the oxidation of the linoleic acid. So in the steril control with manganese, the oxidation of linoleic acid was inhibited almost during the first 2 days of incubation (Table 1). After it increased and reached an important level after 8 days of incubation. Manganese at high concentration can dismute superoxide radical (Engesser and Hammes 1994), this can explain why in the MCMn media , oxidation is delayed the first two days.

Effect of staphylococci in the oxidation of substrates in media MC : The *Staphylococci* were inoculated in MC media with the 4 different susbstrates. All the strains had no effect on the oxidation level recorded in the steril control with pork fat or oleic acid (data not shown). But all the species inhibited sharply the oxidation of linoleic acid (C18:2) (Table 2). This inhibition lasted during all the incubation period i.e. 20 days (data not shown). With the linolenic acid (C18:3), after 5 days of incubation, the inhibition of the oxidation was not so pronounced and also it was dependant of the bacterial strains (Table 2). *S. xylosus* 831 and the two strains of *S. carnosus* 833, 836 were the most effective to fight against oxidation., the inhibiton was between 35 to 40 %. The other species, *S. xylosus* 16, *S. saprophyticus* 852 *and S. warneri* 863, did not reduce or reduced weakly the oxidation (Table 3). After 5 days of incubation, all the strains had difficulties to limit oxidation of C18:3 (data not shown)

It is difficult to compare our results to those of the literature because there are few studies. However Lilly *et al.* (1970) and Alford *et al.* (1971) had shown that *Staphylococcus aureus* inhibited the oxidation of fresh lard and decreased the concentration of peroxides in rancid lard.

Oxidation of linoleic acid in the presence of lactic acid bacteria in the media MC, MCMn : The manganese also influenced the behaviour of the lactic acid bacteria (Table 3). So in the media without manganese, only *Lactobacillus plantarum* inhibited clearly the oxidation of the linolenic acid. This inhibition lasted during the first 8 days of incubation, then after 15 days the oxidation of linoleic

was identical to those of the control (data not shown). For the other lactic acid bacteria, no clear inhibition was recorded during all the incubation period. With manganese in the media, not only *L. plantarum* limited the oxidation, but also *L. sake*, *L. curvatus* and *P. pentosaceus* (Table 3). The two species of *Carnobacterium* did not show again a pronounced inhibition of the oxidation of linoleic acid.

A non heme catalase which is a manganese enzyme is described for *L. plantarum* and *P. pentosaceus* (Engesser and Hammes, 1994), this enzyme will contribute to the limitation of the oxidation of linoleic acid. However, *L. sake and L. curvatus* did not synthesis non-heme catalase (Hammes and Knauf, 1994) and they delay the oxidation of linoleic acid only in the media with manganese. Also *L. plantarum* had an antioxidant activity in media devoid of manganese.

CONCLUSION

In conclusion, no strain either *Staphyloccci* or LAB had prooxidant activity. The different species of *Staphylococci* limited oxidation of unsaturated free fatty acids. *S. carnosus* and *S. xylosus* were the most effective species. Concerning the lactic acid bacteria, the two *Carnobacterium* species had no antioxidant properties. For the other lactic bacteria, their antioxidant activity was influenced by the presence of manganese. With manganese, *L. plantarum*, *L. sake*, *L. curvatus* and *P. pentosaceus* limited the oxidation of linoleic acid. In the future, it will be very important to characterize the anti-oxidant properties of the species used as starter cultures. So, catalase and superoxide dismutase activities are studied for *S. carnosus* and *S. xylosus* (Barrière *et al.*, 1998).

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Times (days)	0	2	5	8	15	20
MC pork fat	3.12	2.23	3.280	3.68	3.27	3.27
MC C18:1	3.21	3.73	3.73	3.67	2.32	2.19
MC C18:3	11.26	31.26	48.18	35.16	32.77	32.01
MC C18:2 (trial 1)	5.53	13.65	23.50	19.80	13.22	13.19
MC C18:2 (trial 2)	4.71	10.20	26.15	25.04	18.00	ND
MCMn C18:2 (trial 2)	3.63	3.66	15.71	30.47	34.00	ND

Table 1 : Kinetic evolution of the oxidation of the substrates prok fat, oleic acid C18:1, linolenic acid C18:3 and linoleic acid C18:2 in the steril control media MC or MCMn (Oxidation is expressed in µmoles/g of substrate, ND : not done)

Staphylococci	C	833	836	831	873	16	863	852
MC C18:2 (trial 1)	23.50	1.73	2.03	2.76	3.34	3.76	3.90	3.76
MC C18:3	48.20	30.90	31.80	28.50	36.90	43.10	40.60	48.00

Table 2 : Effect of the staphylococci on the oxidation of linoleic acid (C18:2) and linolenic acid (C18:3) after 5 days of incubation (Oxidation is expressed in µmoles/g of substrate, C: control steril sample, Strains : *S. carnosus* 833, 836 ; *S. xylosus* 831, 873, 16; *S. warneri* 863 ; *S. saprophyticus* 852)

Lactic Acid Bacteria	Control	*C. divergens*	*C. piscicola*	*L. sake*	*L. curvatus*	*L. plantarum*	*P. pentosaceus*
MC C18:2 (trial 2)	26.15	18.17	19.84	22.29	23.00	9.62	19.31
MCMn C18:2 (trial 2)	30.47	20.33	20.68	5.24	7.40	5.86	3.86

Table 3 : Effect of the Lactic acid bacteria on the oxidation of linoleic acid (C18:2) after 5 days of incubation for the media MC and 8 days of incubation for the media with manganese MCMn (Oxidation is expressed in µmoles/g of substrate)