Influence of plant enzyme inactivation or sterilization on lipolysis in ensiled alfalfa

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Introduction High intake of polyunsaturated fatty acids has been proved to increase their concentration in milk (Lawless et al., 1998) and, consequently be beneficial to human health (Simopoulos, 2001). Therefore, there is increasing interest in the changes of fatty acid composition in herbages and forages after ensiling. Like the extensive proteolysis in ensiled forage, especially in alfalfa silage, numerous studies have reported that lipolysis in silages is also a very extensive process (Van Ranst et al., 2009). It was proposed that lipolysis of forages during ensiling may be caused by both microbial and plant enzymes activation (Elgersma et al., 2003). However, whether the lipolysis during ensiling is mainly caused by microbial or plant lipases is still not clear. Hence, the objectives of the present work were to evaluate changes of fatty acid composition of alfalfa forage after ensiling; and to clarify the importance of microbial and plant enzymes activation on lipolysis of forages during ensiling.

Materials and Methods Third-cut alfalfa (*Medicago sativa*. L. cv. Forerunner) was randomly harvested at late bud to early bloom, leaving a stubble of 10 cm. Fresh forage samples were immediately taken into laboratory, chopped into about 1 to 2 cm length using a paper-cutter. Chopped forages from each experimental plot were then assigned to one of the following treatments: 1) untreated (sterilized water); 2) a commercial inoculants (LaLSiL Dry, manufactured by Lallemand, Montréal, Québec, Canada) after treatment of γ-ray irradiation at a dose of 25 kGy for 2 h; 3) LaLSil Dry after autoclaving treatment at 121 ºC, 115 MPa for 15 min. Before ensiling, DM contents of all treatments were adjusted to about 320 g/kg FW. Five mini-silos were made for each treatment by following the method described previously (Ding et al., 2013), and all mini silos were prepared in an aseptic operating board. Mini silos from each treatment were opened at 40 days of ensiling and immediately frozen (−80°C) in sealed plastic bags until further chemical analysis for fatty acid composition (Ding et al., 2013).

Results and Discussion The concentration of total fatty acid after ensiling decreased 43% in the control silage and 28% in the γ-ray treated silage, but did not change in the autoclave treated silage (Table 1). Among the major fatty acids (C16:0, C18:2n-6, C18:3n-3), a considerable increase ($P < 0.05$) was observed in proportion of C16:0 in the control silage as compared with fresh alfalfa; conversely, decreases in proportions of C18:2n-6 and C18:3n-3 occurred ($P < 0.05$). However, similar concentration of C16:0 in fresh forage, control silage and the silage treated with autoclave was observed. Besides C12:0, C14:0, C15:0, C18:0, cis-9 C18:1, C20:0, and C24:0, silage treated with γ-ray radiation at ensiling had smaller proportion of C16:0 and greater proportions of C18:2n-6 and C18:3n-3 ($P < 0.05$) than the control silage. Proportions of C16:0, C18:2n-6, C18:3n-3 and the other detected fatty acids (except for proportion of C15:0) did not differ between fresh forage and autoclave treated silage ($P > 0.05$). The above results indicate that the total fatty acid content and the $C_{18:3n-3}$ concentration in silage treated with plant enzymes inactivation plus inoculation of lactic acid bacteria was similar to that in fresh forage. Plant enzymes inactivation also inhibited the

reduction of $C_{18:2n-6}$ and the increase of $C_{16:0}$ proportions during ensiling. Based on comparison of the total fatty acid contents in fresh alfalfa and in silages treated with γ-ray irradiation and autoclaving, it can be deduced that plant enzymes was the major contributor to the lipolysis during ensiling. The deduction can also be reflected by the changes in proportions of $C_{18:3n-3}$ in the above mentioned three treatments or by the $C_{18:3n-3}$ concentrations in these treatments.

Table 1 Total fatty acid (FA) content (mg/g of DM), and FA composition (g/100 g of total FA) of fresh, wilted, and ensiled alfalfa after 40 d of ensiling without physical treatment and treated by γ-ray radiation or autoclave before ensiling

	Forage before ensiling		Silages			SEM
	Fresh	Wilted	Control	γ-ray treated	Autoclave treated	
Total FA	29.57^{c}	27.56^{c}	16.82^{a}	21.29^{b}	29.20^{c}	1.214
$C_{12:0}$	0.07^{a}	0.09^{ab}	0.17^{c}	0.12^{b}	0.09^{ab}	0.006
$C_{14:0}$	0.19^{a}	0.25^{b}	0.40^{d}	0.31^{c}	0.21^{ab}	0.012
$C_{15:0}$	0.17^{ab}	0.13^{a}	0.37^{c}	0.33^{c}	0.23^{b}	0.013
$C_{16:0}$	18.65^{a}	18.77^{a}	31.57^{c}	27.06^{b}	20.28^{a}	0.737
$C_{16:1}$	1.04^{a}	0.94^{a}	1.55^{b}	1.48^{b}	1.06^{a}	0.037
$C_{18:0}$	2.91^{a}	3.16^{a}	5.08^{c}	4.30^{b}	3.22^{a}	0.127
$C_{18:1}$ *cis*-9	1.73^{b}	1.10^{a}	1.82^{b}	1.64^{b}	1.35^{a}	0.044
$C_{18:1}$ *trans*-11	0.06^{ab}	0.00^{a}	0.05^{ab}	0.31^{c}	0.14^{b}	0.020
$C_{18:2n-6}$	19.66^{c}	17.71^{b}	15.34^{a}	16.56^{ab}	17.34^{b}	0.219
$C_{18:2}$ *trans*-9, *trans*-15	0.00^{a}	0.00^{a}	2.41^{c}	1.02^{b}	0.75^{ab}	0.154
$C_{18:3n-3}$	54.53^{c}	56.93^{d}	37.85^{a}	45.36^{b}	53.92^{c}	1.016
$C_{20:0}$	0.29^{a}	0.28^{a}	0.63^{b}	0.51^{b}	0.33^{a}	0.024
$C_{22:0}$	0.50^{a}	0.48^{a}	0.86^{b}	0.80^{b}	0.46^{a}	0.034
$C_{24:0}$	0.20^{a}	0.21^{a}	1.88^{c}	1.10^{b}	0.57^{ab}	0.109

$^{a-d}$Within a row, means without a common superscript letter differ ($P < 0.05$).

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