Formation of volatile organic compounds (VOC) during the course of **maize fermentation** depending on **delayed sealing** and **silage additive**

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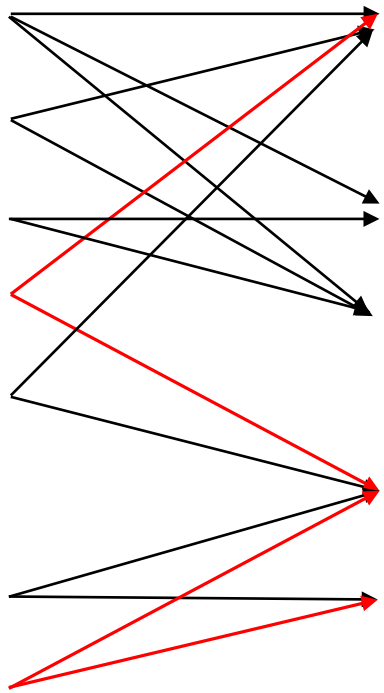
Introduction



VOC	Produced by
Acetic acid	**Heterofermentative LAB**, *Lactobacillus buchneri*, *Lactobacillus diolivorans*
Propionic acid	
Methanol	**Enterobacter**
Ethanol	**Clostridia**
1-Propanol	*Clostridium butyricum*
	Yeasts
Aldehydes*	**Homofermentative LAB**
Esters*	

Ethyl acetate (EA)
Ethyl lactate (EL)
Propyl acetate (PA)

* Additionally by chemical synthesis

Introduction

VOC

Acetic acid

Propionic acid

Methanol

Ethanol (EtOH)

1-Propanol

Aldehydes

Esters

- Ethyl acetate (EA)
- Ethyl lactate (EL)
- Propyl acetate (PA)

Influenced by

Ensiling material

Ensiling conditions

- **Delayed sealing**
- **Silage additives**

Aim of study:

Investigation of **formation** and **accumulation** pattern of **VOC** during **course of fermentation**, **yeast** development and **dry matter losses**

Lab-scale trial with forage maize (26.8 % DM)

- three replicate 1.5-L jars per treatment
- packing density 195 kg DM m^{-3}
- opening after **3,7,16,34,62 and 142** days of storage (22° C)
- Treatments:

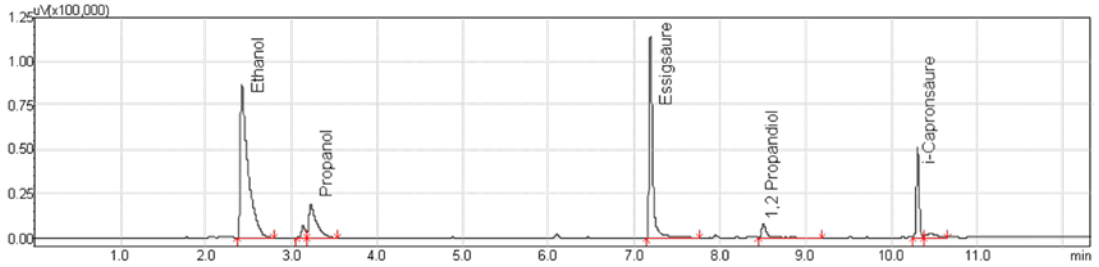
CON without silage additive

SA chemical additive 2 L t^{-1}:
257 g L^{-1} sodium benzoate,
134 g L^{-1} potassium sorbate,
57 g L^{-1} ammonium propionate

- Either immediately sealed (prompt) or sealed with a 24 hour delay (**DEL**)

Analysis of 72 silage samples for:

- DM, DM losses
- pH
- Fermentation acids (GC)
- Alcohols (GC)
- Esters (GC)
- Yeasts



Statistical analysis (procedures MIXED and REG of SAS, 9.4)

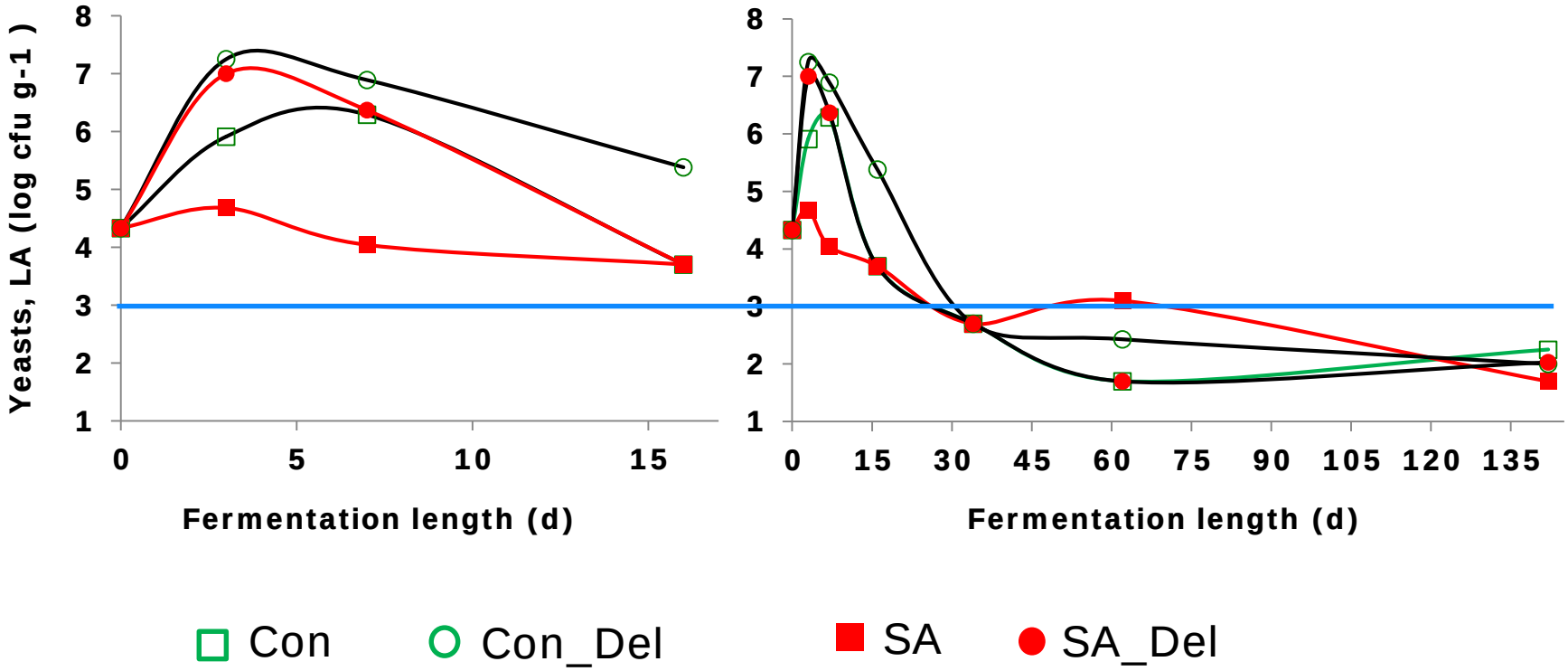
Experimental factors: Fermentation length (F)
Sealing time (S)
Additive (A)

Results

Yeast development

during

early phases of fermentation **entire fermentation process**

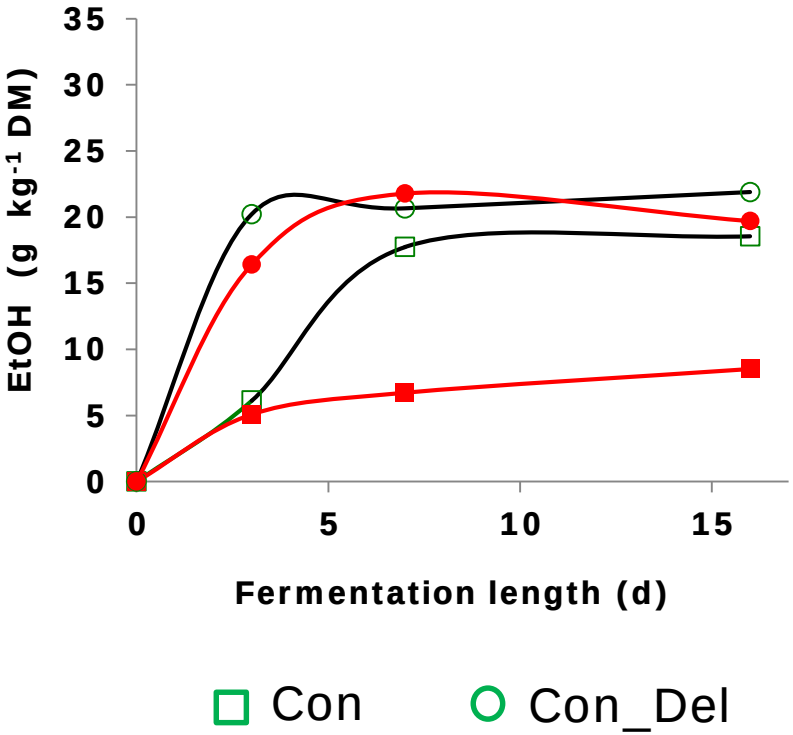


Results

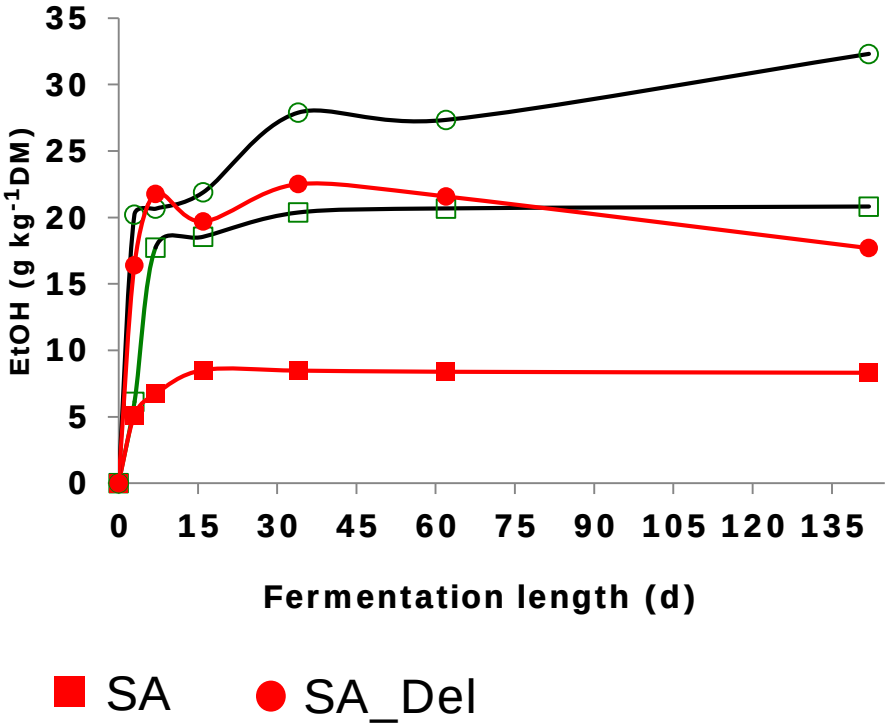
Ethanol formation

during

early phases of fermentation



entire fermentation process



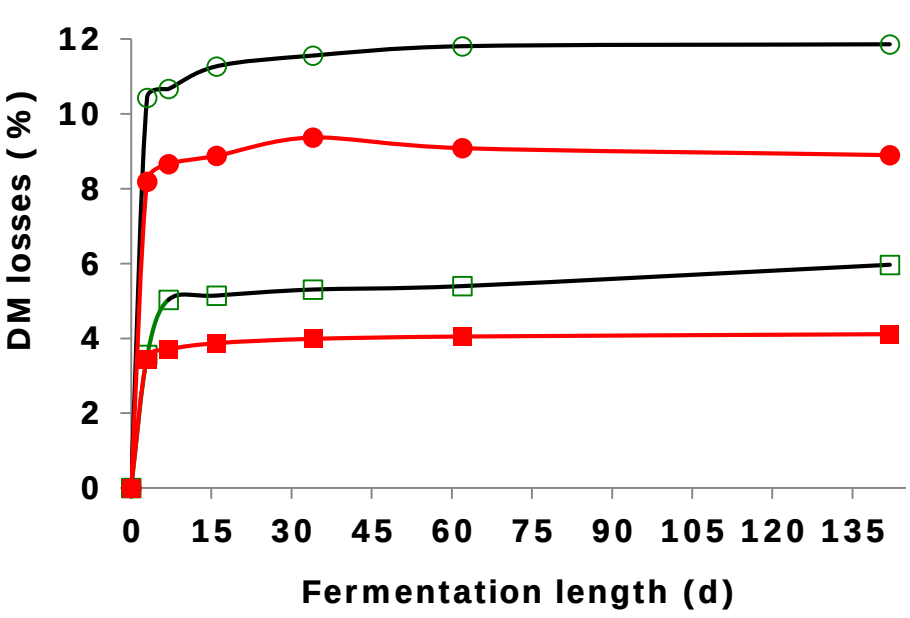
Results

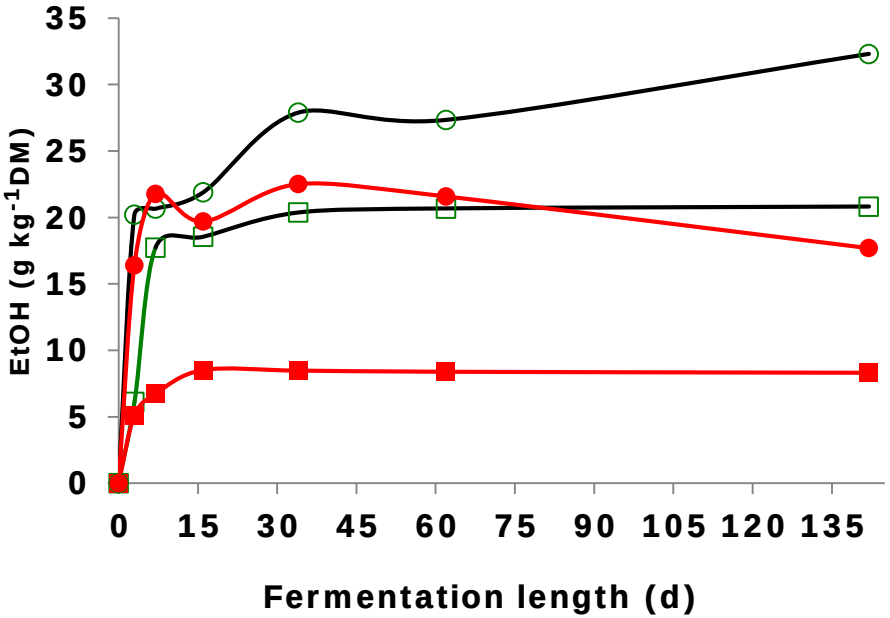
Ethanol formation

during

and DM losses

entire fermentation process



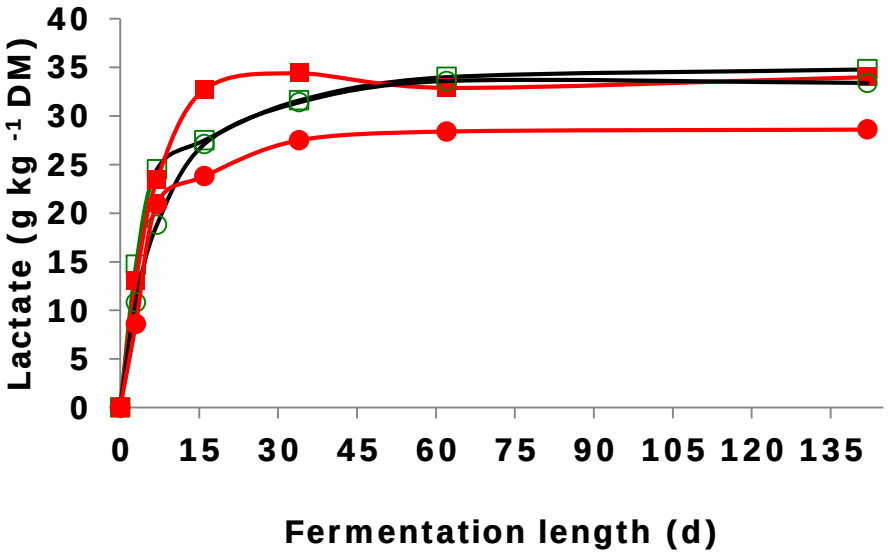


Con Con_Del SA SA_Del

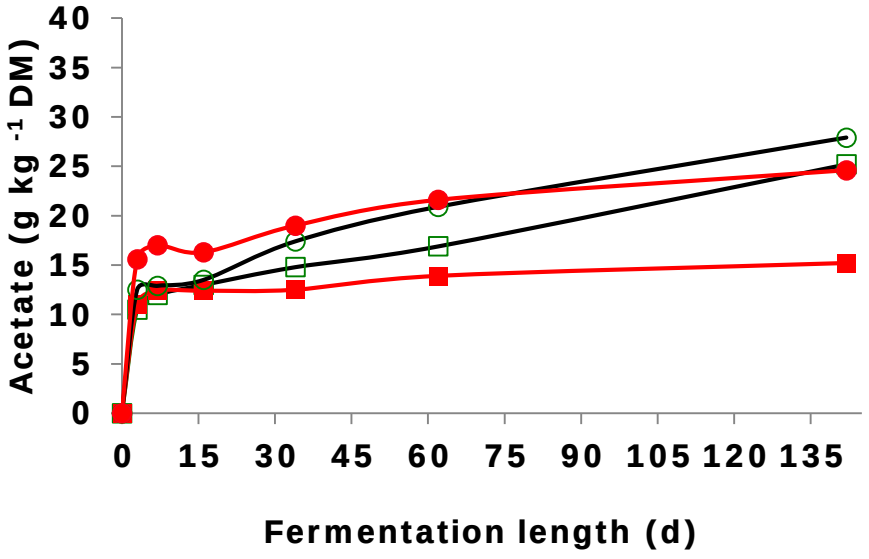
Results

Fermentation acids

Lactic acid



Acetic acid



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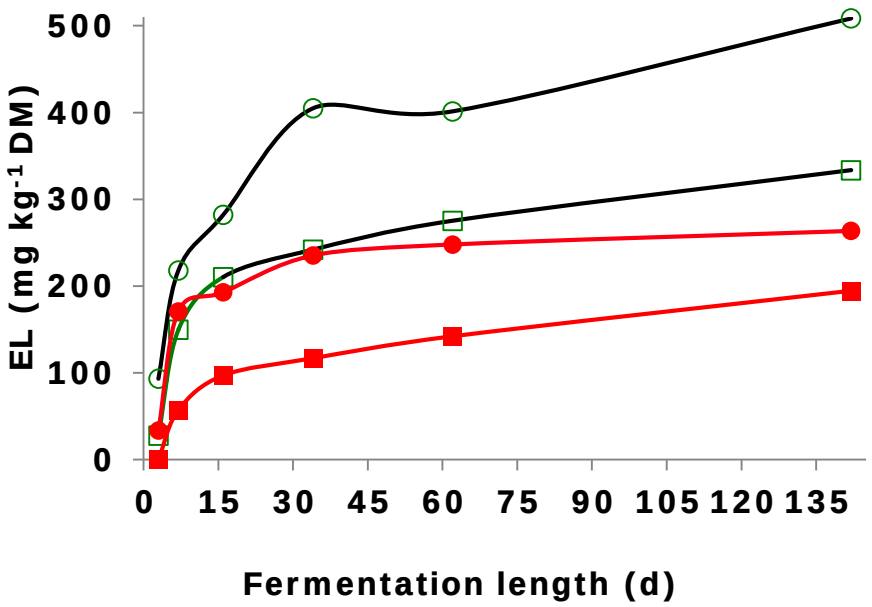
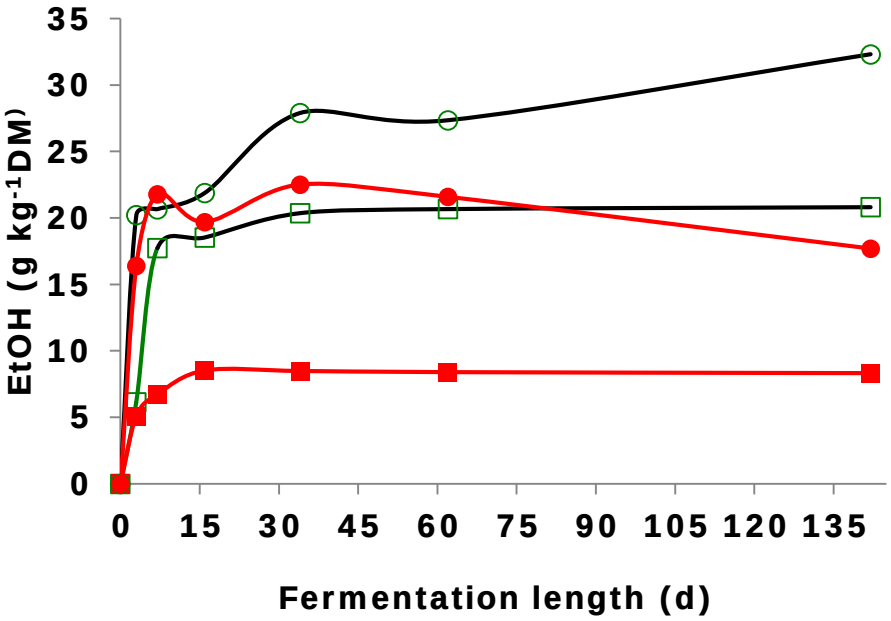
Results

Ethyl esters

Ethyl lactate (EL)



Ethanol (EtOH)



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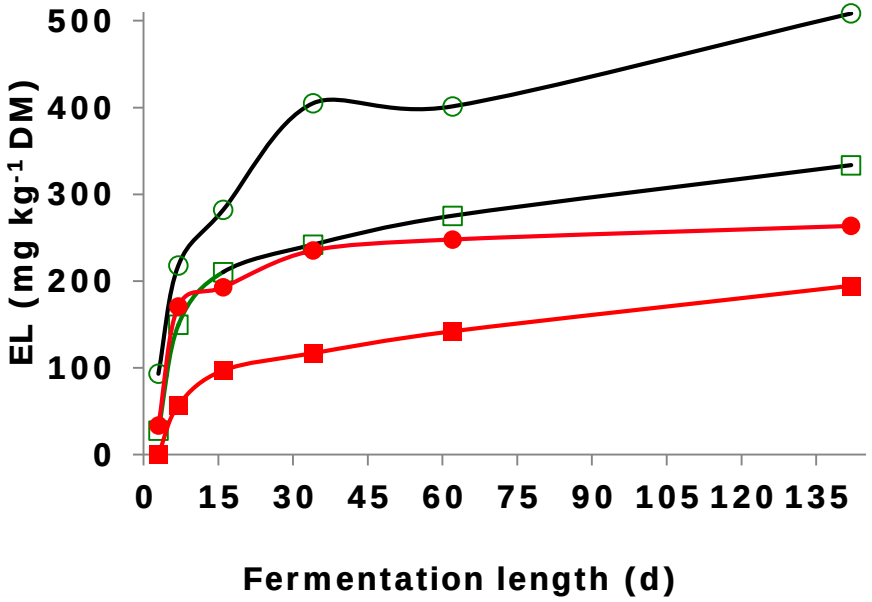
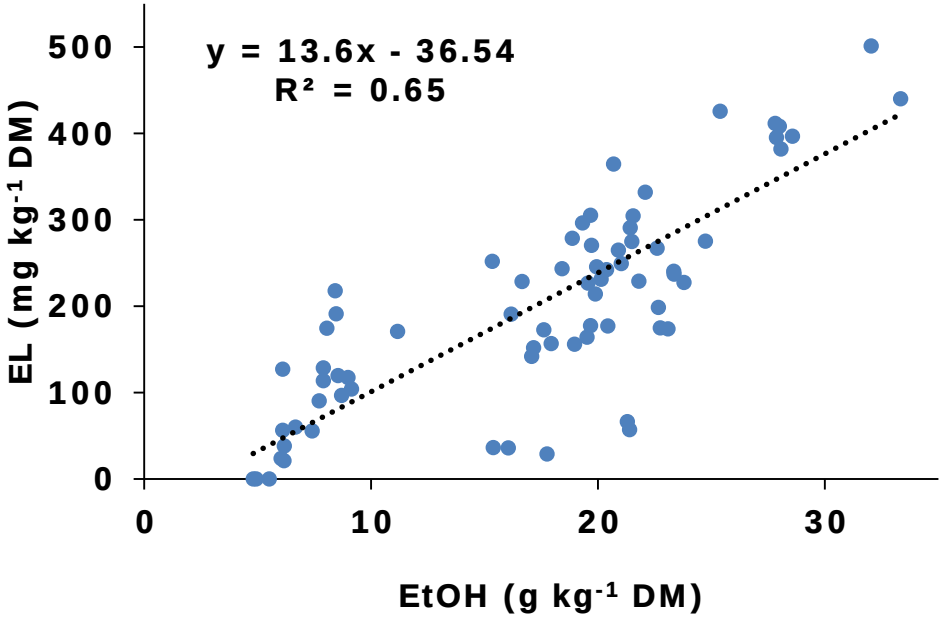


Regression EtOH_EL



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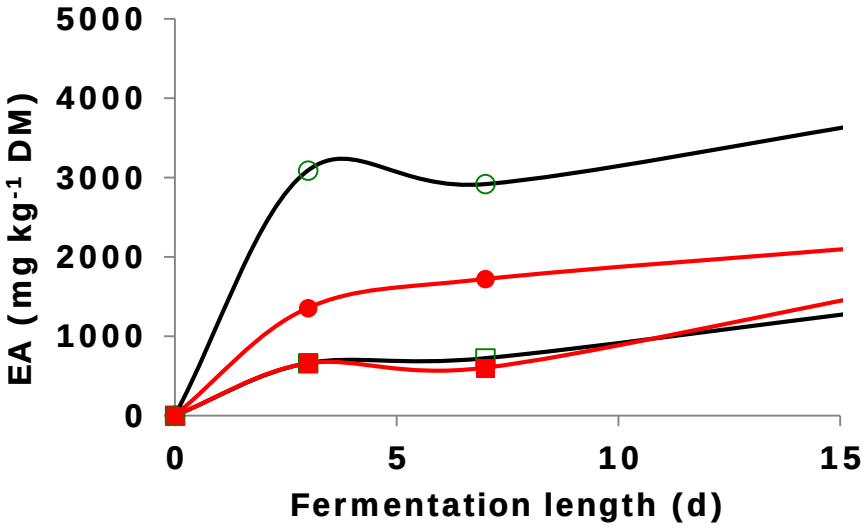
Results

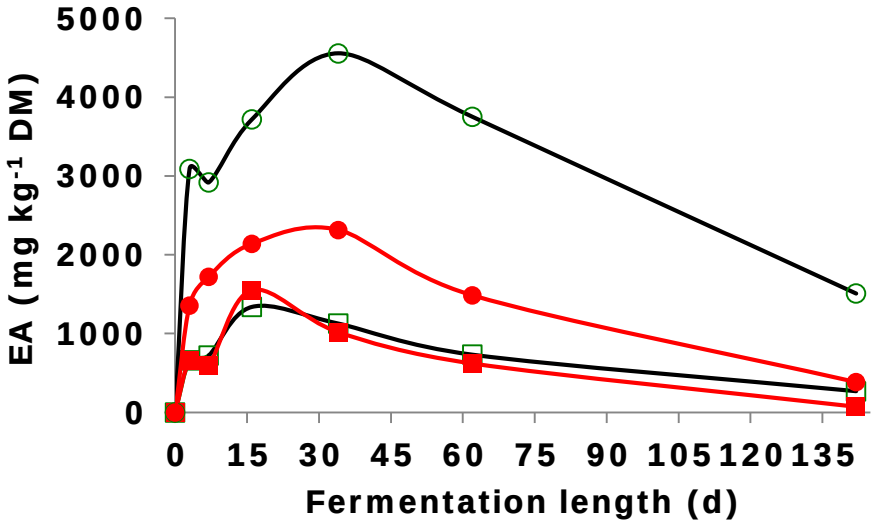
Ethyl esters

Ethyl acetate (EA)

early phases of fermentation



entire fermentation process



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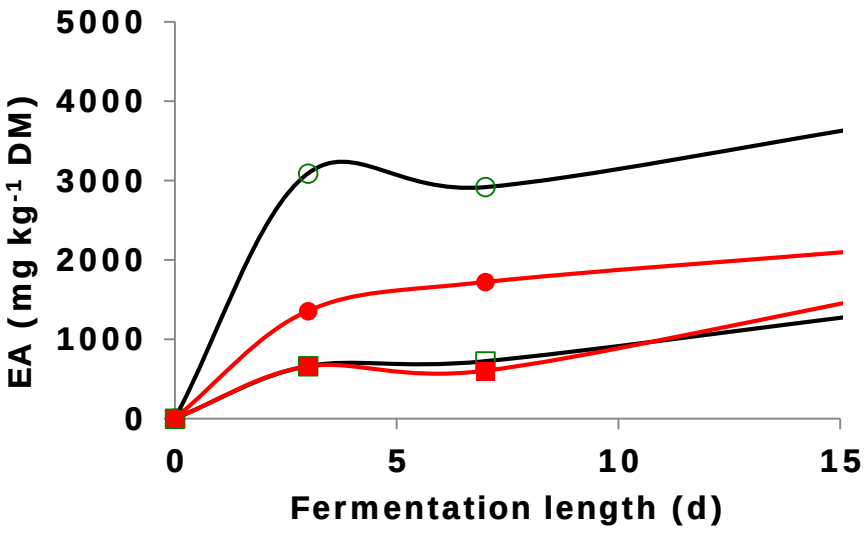

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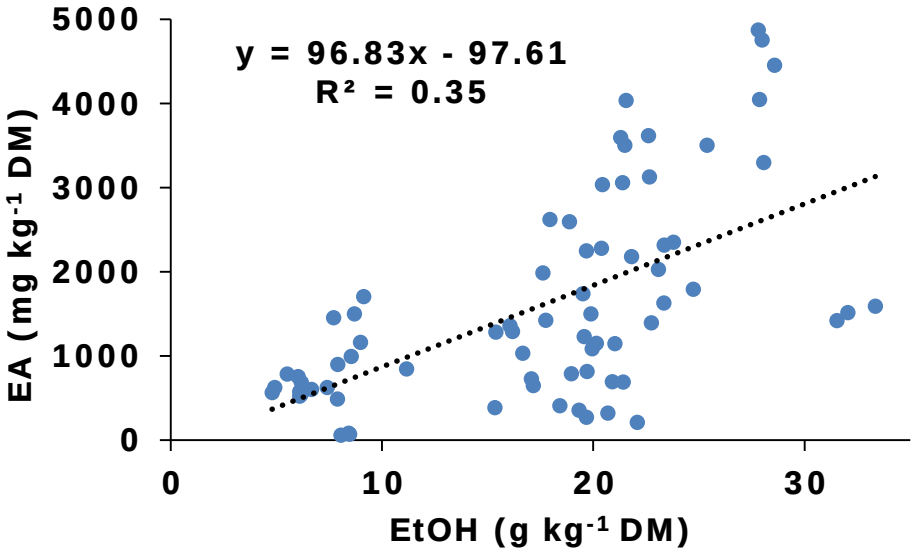

Con Con_Del SA SA_Del

Discussion

Decline in ethyl acetate (EA)- concentration! Why?

pH: in all samples < 4.0

↷ no effect on reaction equilibrium

Formation: - Yeasts are able to produce EA from ethanol and acetyl-CoA with alcohol acetyltransferase (AAT)

- Esterases, found in yeasts, are able to hydrolyze esters

↷ Balance depends on many factors like pH, temperature, carbohydrates, yeast strain, ...

Vapour pressure: 98 mbar (EL 2 mbar)

↷ EA may have collected in the headspace of the jars and be released whenever a lid-lifting overpressure had built-up (in contrast to EL)

Discussion

Ester synthesis

1. Assumption:

   The pathway, chemical or biochemical, differs between the two esters EA and EL

2. EA

   Certain yeast species can produce EA

   (Kruis et al, 2017, Fredlund, 2004; Yoshioka& Hashimoto 1980, 1983,1984, Nordström, 1966)

In this study: Dramatic increase of yeasts and EA during the first 3 days of storage, especially in delayed silages

EA directly by biochemical pathway
EL by chemical pathway

Conclusions

- Delayed sealing stimulates yeast activity
  resulting in
  excessive ethanol production
  formation of ethyl acetate
  and high DM losses

- The correlation between the concentrations of ethanol and ethyl esters vary depending on the type of ester indicating that pathways differs depending on ensiling conditions

- Additive use can partially alleviate the detrimental effects of delayed sealing regarding yeast development, DM losses and VOC formation

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Thank you for your attention!