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RESEARCH ARTICLE

RESEARCH CONCERNING THE INFLUENCE OF LINOLEIC ACID ADDITION IN WORT COMPARING WITH WORT AERATION

Camelia BONCIU*

„Dunarea de Jos" University Galati, Food Science and Engineering Faculty
Domneasca Street, no. 111, tel. 0745914376, e-mail cbonciu@ugal.ro

Abstract

It is well known that yeasts need oxygen at the beginning of the fermentation process for the synthesis of essential compounds for cell membrane, like unsaturated fatty acids and ergosterols. Because the yeast needs the oxygen for the multiplication phase, the brewers aerate the cleared wort with 5-8 mg O_2/l. Studies showed that yeast uses oxygen for lipid synthesis needed for cell membrane. It was also observed that yeast is able to use wort's lipids which have generated the idea that, adding lipids in wort, especially unsaturated fatty acids, would be an interesting alternative for wort aeration. It was also demonstrated the negative implication of the fatty acids addition in aroma profile of the fermented wort. Based on these considerations the effect of linoleic acid addition in wort on the fermentation intensity and aroma compounds synthesis have been studied and compared to fermentation dynamics and aroma profile of the beer obtained from aerated wort.

Key words: wort fermentation, aeration, fatty acids, linoleic acid, aroma compounds

Introduction

Brewing yeasts need a certain amount of oxygen at the beginning of the fermentation process. The oxygen requirements are critical and depend of wort quality, sterols and unsaturated fatty acids disposal and yeast type and history. In the past, wort saturation with oxygen was enough, but nowadays brewers ask for wort saturation with pure oxygen. (Briggs *et al.,* 2004)

Most yeast need oxygen for growing. In a study implying 75 yeast strains it was shown that only 23% of the strains could grow in anaerobic conditions on a complex medium supplemented with ergosterols and unsaturated fatty acids. Saccharomyces cerevisiae was one of the exceptions able to rapidly grow at a low oxygen concentration. (Briggs *et al.,* 2004)

Lipids have a structural important role, especially for cell membrane, conferring them special properties given by hydrophobic character of lipids.

Wort aeration control is difficult. An insufficient aeration can lead to an insufficient revitalization of the yeast, growing deficiency and low fermentation rates. An over-aeration may lead to high biomass amount and low ethanol concentration. (Moonjai *et al.*, 2002) Unsaturated fatty acids can be token from wort, but usually worts do not contain enough unsaturated fatty acids. (Moonjai *et al.*, 2000)

* Corresponding author: cbonciu@ugal.ro

That is the reason why wort supplementation with unsaturated fatty acids was suggested as an alternative for wort aeration. Wort supplementations with lipids or wort aeration lead to volatile esters synthesis decrease. Not all unsaturated fatty acids serve like growing factors for yeasts, is showed in a study of Walenga et all. (Walenga and Lands, 1975)

Since 1955 Klein showed that Saccharomyces cerevisiae cells growing in aerobic conditions contain more fatty acids and ergosterols than yeast cells grown in anaerobiosis and that aeration of the cells grown in anaerobic conditions leads to a dramatic rise of the sterol and fatty acids content. In 1960, Bloomfield and Block showed that oxygen is needed for ergosterols synthesis from squalen and for unsaturated fatty acids synthesis.

Cell membranes with a low sterols and fatty acids content are wicked, affecting biochemical processes at this level (for example sugar transportation or nutrient transportation). Negative impact of the fatty acids supplementation on aroma compounds synthesis was demonstrated. By adding fatty acids to the wort before fermentation the ester content of the final beer will be lower, especially for acetate esters.

Esters arise from the reaction between an acyl-CoA and an alcohol. The alcoholic substrate is usually ethanol but other higher alcohols may participate. Acyl-CoA may derive from fatty acids, but, most used is acetyl-CoA formed from the oxidative decarboxylation of pyruvate. The reaction is catalyzed by an alcohol-acetyl transpherase. This enzyme can be inhibited by lipids, especially by unsaturated fatty acids. The most efficient in inhibiting ester synthesis is linoleic acid. (Verstrepen *et al.*, 2003)

Volatile aromatic active esters are the most and important group of aromatic compounds found in beer. They are responsible of the desired fruity aroma, candy and perfume aroma of fermented drinks. In beer there are about 100 different esters which can be grouped in two categories: Acetate-esters – ethyl acetate (solvent), isoamyl acetate (fruity, banana), phenyl ethyl acetate (flowery, roses, honey) and ethyl esters – ethyl caproate (C_6), ethyl caprylate (C_8), ethyl caprate (C_{10}). (Verstrepen *et al*., 2004)

Acetate esters made by the yeasts during fermentation are extremely important for the flavor of the beer. Their presence is wanted in certain amounts and an inadequate fermentation leads to an insufficient amount of esters resulting aroma defects of beer. So, esters regulation is a necessity in brewing industry. Therefore, it is important to know how much the ester content decrease when wort is supplemented with linoleic acid.

In this paper the effect of linoleic acid supplementation on final beer aroma compounds and compared with aroma profile of the beer obtained from aerated wort were investigated.

Material and methods

For the comparison of the aerated wort fermentation with linoleic acid supplemented wort fermentation industrial wort was used, having the following characteristics: original extract 17.36°P, pH 5.35, color 10.9 EBC units, bitterness 51.5 BU, FAN (free amino nitrogen) content 156.3 ppm, polyphenol content 215.5 mg/l.

The wort was corrected to different original extracts by using water for extract dropping and industrial maize syrup for original extract rises. The syrup used for experiment had the following characteristics: dry matter content 70.6%, 69.2°Brix, fructose content 41.8% and pH 4.1. The syrup was obtained by maize starch hydrolysis.

The worts obtained had the characteristics presented in the table 1.

The yeast strain used for fermentation process was industrial slurry yeast and had the following characteristics: viability 91.04%, consistency 34% and pH 4.03. The wort was pitched with $1.5x10^7$ cells/ml.

The linoleic acid solution used for wort supplementation had 60 mg/l concentration in etilic alcohol.

Table 1. *The characteristics of the worts used for experiment.*

Wort characteristics	desired original extract, °P	15	17	21	24	28
pH		5.37	5.35	5.33	5.3	5.3
Color, EBC		9.06	10.9	9.8	9.11	8.35
Obtained original extract, °Plato		15.19	17.36	21.92	25.81	30.09
FAN, mg/l		132.8	156.3	129.6	111.5	89.8
Polyphenols, mg/l		185.5	215.5	189	161	148.5
Bitterness, BU		46.6	51.5	49	44.7	43

The laboratory equipments used were:

- Karl Zeiss Jena Microscope – for cell counting,
- Analytical balance Owalabor type 750.05 – for weighing the samples,
- Shimadzu gas-chromatograph with capillary column Chromopack 7773, length 50 m, liquid phase AT WAX , detectors FID and ECD, mobile phase N_2/H_2 – for the determination of aroma compounds,
- Anton Paar DSA 5000 – for alcohol content and extract determination
- Toledo pH-meter
- CECIL CE 4002 UV-VIS spectrophotometer.

The methods used were:

- Direct counting of microorganisms with Thomas camera,
- Yeast viability using methylene blue method,
- Gas-chromatographic determination of aroma compounds using EBC method,
- Apparent extract determination with Anton Paar,
- Ethanol determination using standardized method SR 13355-3/1999,
- pH determination using pH-meter,
- Free amino nitrogen determination using ninhydrin method,
- Bitterness determination,
- Polyphenol determination.

Results and discussion

The fermentation process was conducted in 500 ml Erlenmeyer flasks containing 300 ml of wort at a constant temperature of 19°C for 184 hours.

The samples were prepared as shown in Table 2. Therefore, samples numbered 1, 2, 3, 4 and 5 were worts supplemented with acid linoleic and not aerated. Each of these samples had a different original extract. Samples numbered 6, 7, 8 and 9 are aerated worts without linoleic acid supplementation. Before fermentation, samples 6 to 9 were aerated with a pure air flow for 3 minutes. 50% ethanol solution was added in these samples for error avoid because of the ethanol content of the linoleic acid solution.

Table 2. *Sample preparation*

Sample	1	2	3	4	5	6	7	8	9
Aerated/non aerated wort	n*	n	n	n	n	a**	a	a	a
Original extract, Plato	~15	~17	~21	~24	~28	~15	~17	~21	~24
50%ethanol added, ml						3.5	3.5	3.5	3.5
Linoleic acid added, ml	3.5	3.5	3.5	3.5	3.5				

* non aerated wort ** aerated wort

The samples were weighed at each 12 hours for the emitted CO_2 determination, thus giving details concerning fermentation intensity. The fermentation dynamics is given in figure 1.

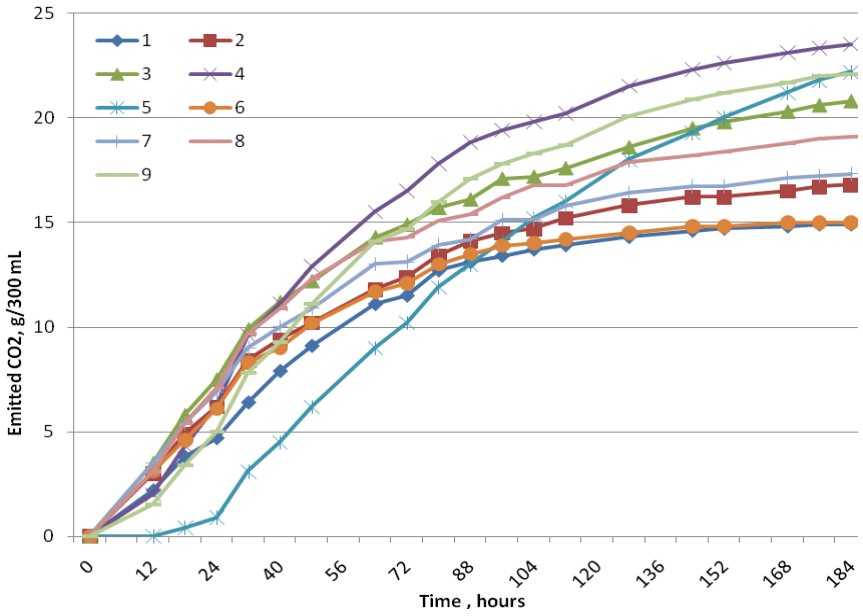

Figure 1. *The fermentation dynamics for the wort samples*

After sample analysis all the obtained results were expressed reporting to a 12°Plato standard wort.

As it can be observed from following figures (figures 2 to 7), samples' color, polyphenol content, free amino nitrogen content, bitterness, apparent extract and alcohol content have a similar evolution in the samples obtained from linoleic acid supplemented wort as in the samples obtained from aerated wort.

Higher differences can be observed in polyphenol content and bitterness at lower original extract of the wort (figures 4 and 5).

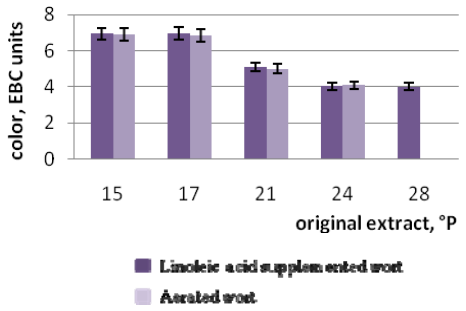

Figure 2. *Color of the samples obtained by aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

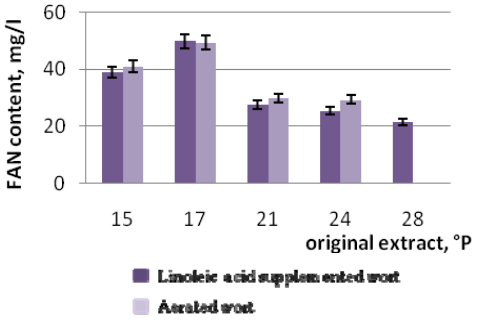

Figure 3. *Free amino nitrogen (FAN) content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

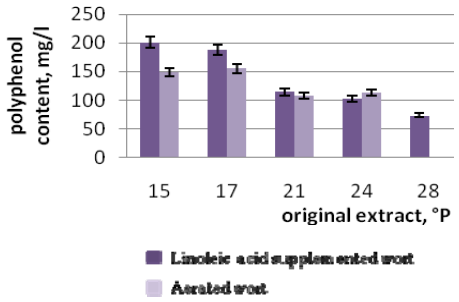

Figure 4. *Polyphenol content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

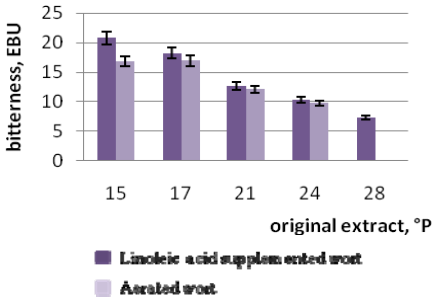

Figure 5. *Bitterness of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

Apparent extract evolution is in accordance with ethanol formation (figures 6 and 7). When wort density is higher, the apparent extract is higher and the ethanol content is lower.

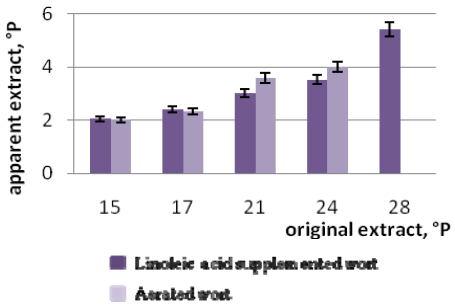

Figure 6. *Apparent extract of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

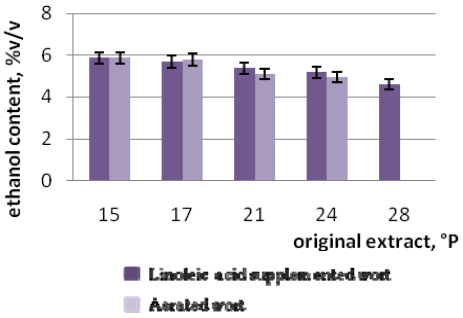

Figure 7. *Ethanol content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

Aroma compounds content is different, depending of samples extract and wort aeration or linoleic acid supplementation.

Therefore, diacetyl content is higher as the original extract of the samples is higher both for aerated wort and linoleic acid supplemented wort as it can be seen from figure 8. In the samples obtained from aerated wort the diacetyl content is higher than in the samples obtained from linoleic acid supplemented wort.

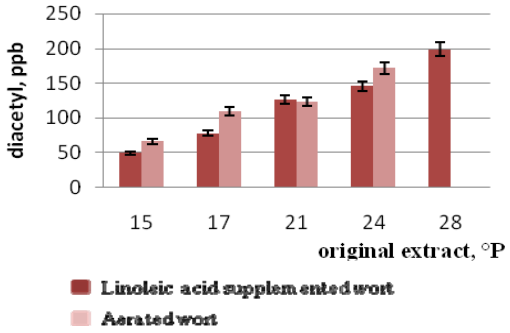

Figure 8. *Diacetyl content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*
ppb – parts per billion

As figure 9 shows, the pentanedione content of the samples is low. For original extract of the sample lower than 20°P the pentanedione content of the sample obtained from aerated wort is higher than in the samples with original extract over 20°P.

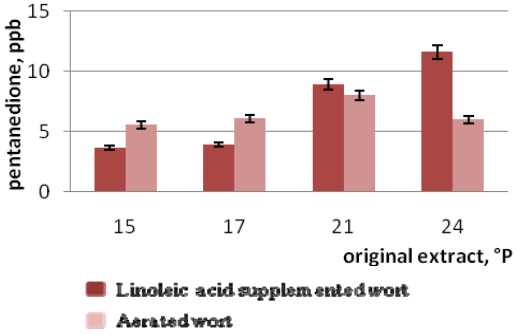

Figure 9. *Pentanedione content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

In the samples obtained from aerated wort it can be easily observed a higher acetaldehyde content than in the samples obtained from linoleic acid supplemented wort – figure 10.

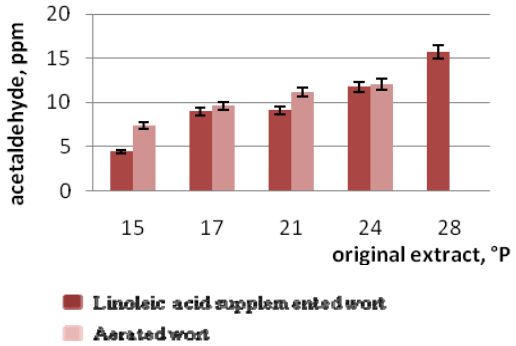

Figure 10. *Acetaldehyde content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*
ppm – parts per million

As shown in figures 11and 12, the esters content of the samples are higher when the wort was aerated, both for isoamyl acetate and ethyl acetate, confirming the results obtained by other specialists. (Moonjai *et al.*, 2000; Moonjai *et al.*, 2002)

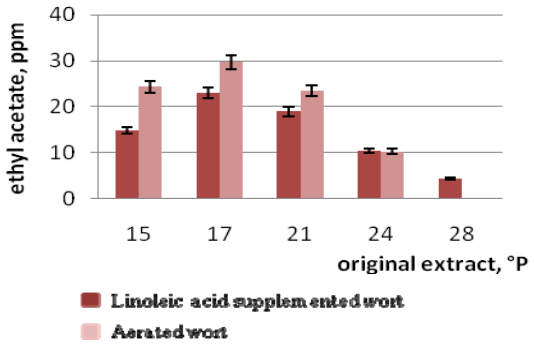

Figure 11. *Ethyl acetate content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

As it can be observed, the difference between esters content for beers obtained from aerated wort and beers obtained from supplemented wort is lower as the original extract of the wort is higher. It is well known that esters production is higher as wort density is higher. This raising it is not observed in our samples, probably due to the dilution of growing factors and of free amino nitrogen content by adding water or syrup to the wort.

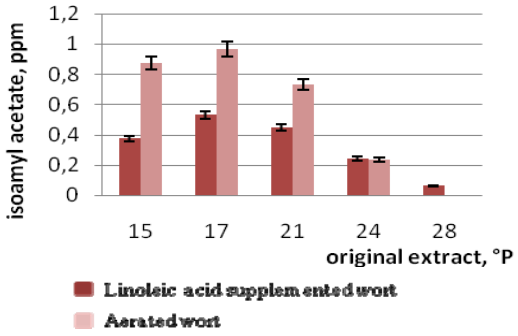

Figure 12. *Isoamyl acetate content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

The higher alcohols content of the samples is higher in the samples obtained from linoleic acid supplemented wort as it is showed in figures 13, 14 and 15. These results were obtained by other researchers too. (Moonjai *et al.*, 2000; Moonjai *et al.*, 2002)

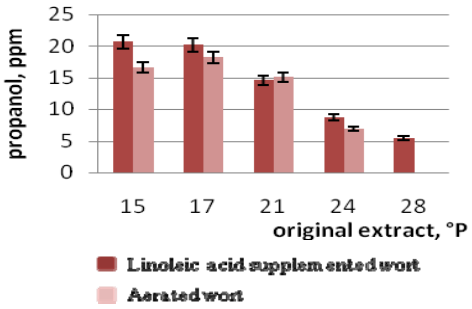

Figure 13. *Propanol content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

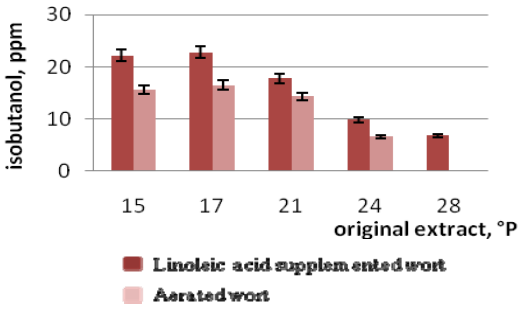

Figure 14. *Isobutanol content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

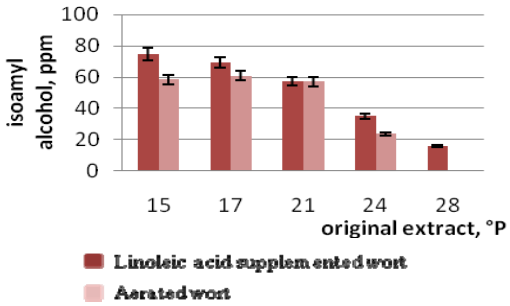

Figure 15. *Isoamyl alcohol content of the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

In the figure 16 is presented the evolution of the higher alcohols and esters ratio. It can be observed that the ratio between higher alcohols content and esters content of the samples is higher when linoleic acid was added to wort before fermentation. The same results were obtained by Moonjai *et al.*, 2000 and 2002.

Figure 16. *Higher alcohols/esters ratio for the fermented samples obtained from aerated and linoleic acid supplemented wort fermentation for different original extract of the wort (15, 17, 21, 24, 28°P)*

Conclusions

Wort supplementation with linoleic acid for aeration avoidance influences final beer aroma. Generally, lower amounts of diacetyl, pentanedione, acetaldehyde and esters were formed and higher amounts of higher alcohols were found.

Wort supplementation with linoleic acid could be an interesting possibility to replace wort aeration before fermentation. This technique can have negative effects on acetate esters and higher alcohols synthesis, and finally on final beer aroma.

However, wort supplementation with linoleic acid has positive effects when high density worts are fermented, when usually a higher amount of esters is formed.

It has been demonstrated that wort supplementation with linoleic acid before fermentation leads to higher alcohols content rising and lower amounts of esters in the final beer.

Linoleic acid addition to wort before fermentation has to be correlated with yeast strain characteristics, with wort composition and nutritional equilibrium of wort's compounds.

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