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Technological Steps and Yeast Biomass as Factors Affecting the Lipid Content of Beer during the Brewing Process

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Knowledge of lipid content and composition in the brewing process enables the quality control of the final product. Lipids have a beneficial effect on yeast growth during fermentation as well as deleterious effects on end-product quality. The lipid content of a beer affects its ability to form a stable head of foam and plays an important role in beer staling. Lipid oxidation during wort production is of great interest because of its effect on beer quality: both lipids and their oxidation products are known to have adverse effects on beer flavor, whereas interactions between lipids and protein films stabilizing the gas bubbles are thought to cause the collapse of foam. In this background, the aim of this research was the characterization of the lipid content during a brewing process for evaluating the influence of both technological steps and yeast biomass in the lipid composition of beer. Lipid contents and their fatty acid profile were evaluated in brewing raw materials, wort, and beer. A high-resolution gas chromatography−flame ionization detector (HRGC-FID) system was used for fatty acid determination in lipid extracts. The results of the present study highlighted that the main technological steps influencing the lipid content in brewing byproduct and beer were clarification in a whirlpool and filtration. Moreover, the presence of metabolically active yeast cells (used as starter culture) were found to have a great influence on the fatty acids composition of lipids.

KEYWORDS: Fermentation; hop; lipids; malt; yeast biomass

INTRODUCTION

One of the most important issues in modern brewing processes is how to produce flavor and foam-stable beer. Beer lipids can adversely affect beer quality, in particular, flavor and foam stability. A stable head of foam and a balanced, refreshing taste are the main characteristics used by consumers for judging the quality of beer. Aging of beer involves changes in flavor impression, particularly due to the oxidation of unsaturated fatty acids, which is related to the musty aroma of stale beer (*1*−*3*).

The damaging effect of lipids on beer foam has been widely documented and easily demonstrated (*4*). Fatty acids having 6−10 carbon atoms had no impact on foam stability, but longer chain fatty acids can destabilize beer foam through a film-bridging mechanism and therefore determine a rapid foam collapse, similar to that currently caused by antifoam systems (*5*).

Due to their negative effects on beer quality, lipid control in brewing process is important for quality improvement of final product. Nonetheless, it is well-known that lipids have a significant influence on both the growth and metabolism of yeast starter cultures. Unsaturated long-chain fatty acids and sterols (in particular, ergosterol) are integral structural components of yeast cell membranes and are essential in the maintenance of plasma membrane fluidity (*6*, *7*).

The aim of this research was the characterization of the lipid content during a brewing process for evaluating the influence of both technological steps and yeast biomass in the lipid fraction of beer. Lipid contents and their fatty acid profiles were evaluated in brewing raw materials, wort, and beer.

EXPERIMENTAL METHODS

Materials. Malt was purchased from a local market (Saplo Pils type, Pomezia, Rome, Italy). Commercial hop pellets (Saaz type, 3.5% α-acid) were purchased from a local market (Pab s.r.l., Pasian di Prato, Udine, Italy). Wort and beer samples (obtained from considered malt and hop) were supplied by CERB (Italian Brewing Research Centre). The dry lager yeast starter culture (belonging to the species *Saccharomyces cerevisiae* and commercially labeled as Saflager W-34/70) was purchased from Fermentis (Oskaloosa, IA).

Two different mashes (A and B) were considered. Mash A was conducted with a decoction mashing system, whereas B (infusion mashing) was a single-temperature mashing system. In the pilot plant, wort samples were collected during different mashing steps: sweet wort, bitter wort

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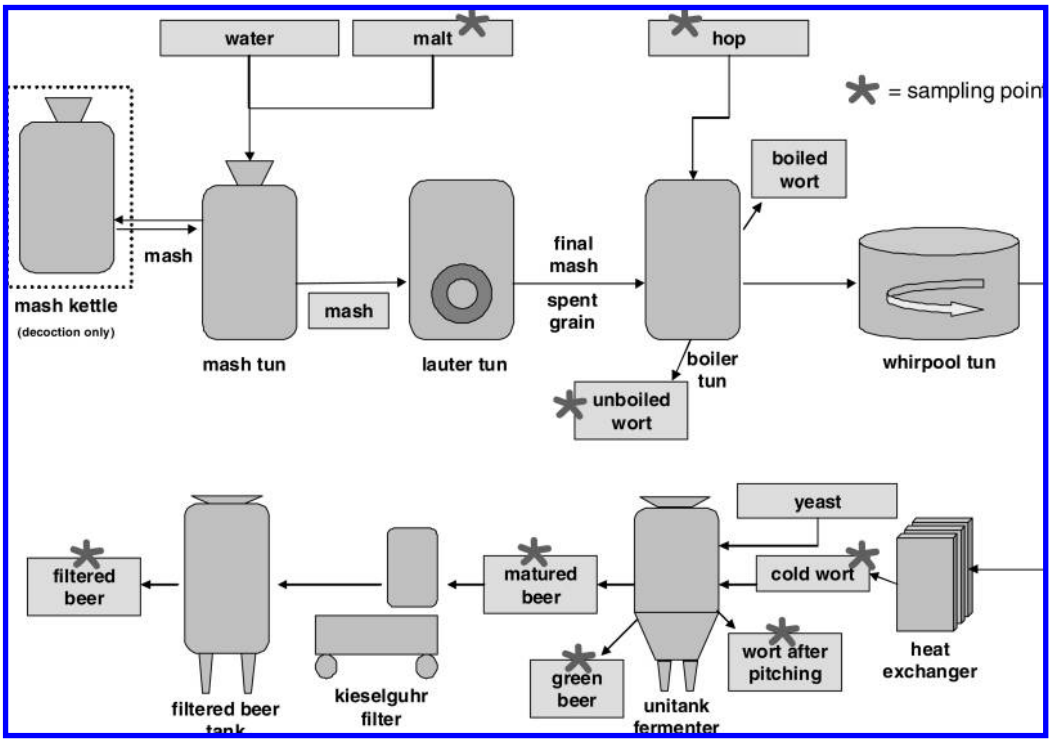

Figure 1. Pilot plant scheme.

(boiled and hopped wort after cooling), and pitched wort at the beginning of fermentation (at 12−18 h from pitching). Beer samples were green beer, matured beer, and filtered beer. The sampling points are reported in **Figure 1**.

Other Materials. HPLC grade water was purchased from Panreac (Barcelona, Spain); HPLC grade methanol, analytical grade petroleum ether, *n*-hexane, chloroform, and HPLC grade diethyl ether were purchased from Carlo Erba (Milan, Italy). Chromatographic purity grade nitrogen, hydrogen, and air were purchased from Linde Gas Tecnici (Perugia, Italy). GC standards (Supelco 37 component FAME MIX) were purchased from Sigma-Aldrich SRL (Milan, Italy). Silica gel plates were purchased from Merck (Darmstadt, Germany).

Mashing. Mashing was performed in a 110 L pilot plant.

For sample A the mashing was carried on by decoction. In decoction process part of the mash is withdrawn and boiled; when it is pumped back, the temperature of the total mash increases. Two vessels were used: mash tun and mash kettle. Mashing was carried out in mash tun at 52 °C for 20 min, and then an amount of almost 20% of mash was transferred to the mash kettle. The remaining 80% of the mash stayed for 60 min in the mash tun at 52 °C. During this time the 20% of mash in the mash kettle was exposed to the following steps: (i) rise of temperature from 52 to 65 °C in 10 min, (ii) held at steady state (at 65 °C) for 15 min, (iii) further increase from 65 °C to boiling temperature for water (100 °C) in 20 min, and (iv) final boiling step for 15 min. After boiling, the mash in the kettle was pumped back to the mash tun in 5 min and, as a consequence, the temperature of mash in the mash tun rose to 65 °C. Another 20% of mash at 65 °C was transferred to the mash kettle and 80% remained in the mash tun at 65 °C for 60 min. During this time the 20% of mash placed in the mash kettle was treated as follows: (i) rise of temperature from 65 to 72 °C in 5 min, (ii) steady state at 72 °C for 20 min, (iii) rise from 72 °C to boiling temperature of water in 20 min, and (iv) final boiling for 10 min. The 20% boiled amount of mash was then transferred to the mash tun. The temperature of the mash tun increased to 76 °C, and then there was a rest at 76 °C for 10 min. After iodometric titration (to verify the whole starch degradation), all of the mash was transferred to a lauter-tun filter.

For sample B the mashing was done by infusion. The temperature profile of mashing was as follows: (i) first steady state at 52 °C for 30 min, (ii) first rise of temperature from 52 to 65 °C in 15 min, (iii) second steady state at 65 °C for 45 min, second rise of temperature from 65 to 72 °C in 5 min, (iv) third steady-state at 72 °C for 20 min, (v) final rise from 72 to 76 °C in 5 min, and (vi) final steady state at 76 °C for 20 min.

Fermentation Process. Pitching was carried out at 11 °C, by using the yeast starter Saflager W-34/70 (bottom fermenting), 1.5 kg/hL (dry weight), approximately 50% (w/w) after aeration with sterile air (8 mg/L).

At the beginning of the primary fermentation, pressure and temperature were settled at 0.1 bar and 11 °C, respectively, until the Plato (the Plato scale expresses the specific gravity as the weight of extract in a 100 g solution at a temperature of about 20 °C; this percentage is designated degrees Plato (*8*)) dropped at 7−8 °P. Afterward, pressure and temperature were increased to 1 bar and 14 °C, respectively, for 2 days (diacetyl pause). The green beer was thus chilled to 0 °C and stored for 2 weeks prior to filtration.

Sample Preparation. Malt samples were finely milled in a Bühler (Bühler AG, Uzwil, Schweiz) grain mill. Hop sample was finely triturated with a pestle and mortar. Wort samples were collected, cooled, freeze-dried, and stored in dark brown glass vessels at room temperature. Beer samples were degassed and concentrated in a rotary evaporator (LABOROTA SEM-320, Resona Technics, Gossau, Switzerland).

Lipid Extraction. The extractions of lipids from malt, hop, yeast biomass, and mashing samples were performed in a Soxhlet with petroleum ether at 60 °C for 9 h runs (*6*, *10*). The yeast biomass was washed with water and 0.1% $NaHCO_3$, followed by centrifugation at 3000 rpm for 5 min before extraction (*11*).

The Bligh and Dyer method (*12*) was used for the extraction of lipids from beer and yeast biomass samples.

Bligh and Dyer Method. Fifty milliliters of concentrated beer was used for Bligh and Dyer extraction. One hundred and eighty milliliters of a 1:2 (v/v) mixture of chloroform/methanol was used for the extraction of lipids from 50 mL of sample under shaking conditions obtained using an ultraturrax. Sixty milliliters of chloroform was then added, and the mixture was vigorously stirred. Finally, 60 mL of water was added. Extraction was repeated four times. The extracts were pooled and evaporated to dryness under N_2 in a rotary evaporator. Lipids were dissolved in 10 mL of chloroform/methanol (1:1; v/v), divided into 2.5 mL aliquots in test tubes, evaporated to dryness under N_2, and stored at −20 °C under N_2 before analysis. The samples were used to determine fatty acid profile and lipid class content.

Separation of Lipid Classes. The dried extracts were redissolved in chloroform/methanol (1:1 v/v). The samples of extracted lipids were then

spotted onto silica gel plates (Kieselgel 60, 0.25 mm) from Merck (Darmstadt, Germany). A solvent mixture consisting of petroleum ether, diethyl ether, and formic acid (70:30:1; v/v/v) was used for separate triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids (FFAs), and polar lipids (PLs). Different lipid classes were highlighted by spraying with 2′,7′-dichlorofluorescein and viewing under UV light, scraped off for their determination.

Preparation and Analysis of Fatty Acid Methyl Esters (FAMEs). The lipid extracts were transesterified by treatment with methanol/KOH solution (*13*), and the resulting FAMEs were injected into the HRGC-FID system. Major fatty acids were identified by comparing their retention times with those of commercial standards (Sigma, St. Louis, MO).

Gas Chromatography. An Agilent model 6850 gas chromatograph equipped with a FID, a capillary inlet system, a DB-23 (60 m × 0.25 mm × 0.25 μm) column, and a model Maestro MPS 2XL multipurpose sampler with a 10 μL syringe was employed (Gerstel Inc., Baltimore, MD). The programmed oven temperature was as follows: 130 °C for 1 min, raised from 130 to 170 °C at 6.5 °C/min, raised from 170 to 215 °C at 2.75 °C/min, 215 °C for 12 min, raised from 215 to 230 °C at 40 °C /min, held at 230 °C for 3 min. The carrier gas (H_2) flow rate was 1.7 mL/min. The split ratio was set at 50:1. The temperatures of the injector and detector were 270 and 280 °C, respectively. Peak areas were measured by using an Agilent MSD Chemstation for HRGC-FID.

Statistical Analysis. All data were analyzed using SigmaStat (version 3.1, Jandel Scientific, San Rafael, CA) software to perform the appropriate statistical tests. Comparisons of the different matrices were made by one-way repeated measures analysis of variance, and the results obtained were further analyzed by using the Holm−Sidak test.

RESULTS AND DISCUSSION

The results for lipid content (as concentrations) and fatty acid profile for raw materials (malt and hop), yeast biomass, wort, and beer, for both mashes A and B, and a comparison between the decoction and infusion mashing are reported and discussed. Afterward, the results are reported in terms of mass balance highlighting the trend of lipid fraction in the whole brewing process.

Malt Lipids. The lipid content of malt was 1.52±0.03 g/100 g (dry weight basis). The moisture content of malt was 6.95 ± 0.03%. Malt lipids contained 70% of TAGs, 10% of DAGs, 4% of MAGs, 7% of FFAs, and 9% of PLs. The fatty acid profile of lipid extract is reported in **Table 1**.

Fatty acids with 8−22 carbon chain lengths were found in malt lipids. The major fatty acids were linoleic, palmitic, oleic, and linolenic acid.

Hop Lipids. The lipid content of hop was 5.71%. The total extract of hop was 13.22±0.03 g/100 g (dry weight basis). Hop extract contained 44% of lipid; the remaining part was total resins (TLC determination, data not reported). The fatty acid profile of the lipid extract is reported in **Table 1**.

The main fatty acids in hop were linoleic, linolenic, lauric, palmitic, capric, and arachidonic acid.

Yeast Lipids. The content of total lipids of the yeast biomass (harvested via centrifugation after fermentation) was 2.15% ± 0.01 g/100 g (dry weight basis). The fatty acid composition of lipids extracted from the yeast biomass is reported in **Table 1**.

Wort Lipids. Table 2 reports the lipid concentration of wort samples (sweet wort, bitter wort, and pitched wort at the beginning of fermentation) for decoction mash A, whereas **Table 3** reports the lipid concentration of wort samples for infusion mash B.

In both mashing systems (decoction and infusion), the lipid content of bitter wort was lower than that observed in sweet wort. Bitter wort is produced from sweet wort through the technological operation of hopping, boiling, and clarification in a whirlpool. A few lipids were introduced with hop (300 g of hop, containing 5.7% of lipids, was utilized). Some lipids originating from malt and hop and initially dispersed into the wort were lost during the boiling and clarification of wort, carried down with the trub, or hot break, and deposited at the bottom of the whirlpool (*14*). Comparing the lipid content of sweet wort samples for mashes A and B, we can emphasize that the infusion mashing system allows obtaining sweet wort characterized by lower lipid content than the decoction system. This behavior could be due to the peculiarity of the infusion and decoction mashing: in the first system all phases are developed in the mash tun, determining the intimate mixing of the ground material with hot water; the mash never reaches the boiling temperature. In the second system the malt is ground more finely than in the infusion process and mixed with water at lower temperature; however, portions of the mash are initially taken out and thus boiled. In the final phase, such portions are pooled with mash tun: this determines a gradual rise of the temperature of the entire mash. Due to the boiling treatment, cell walls of the grains are destroyed; this allows easier access for the hydrolytic enzymes to their substrates (lipases included). The efficiency of decoction mashing is generally higher for the infusion system and allows the highest yield to be obtained from the raw materials (*15*).

Table 1. Fatty Acid Profile[a] of Malt, Hop, and Yeast Biomass Lipid Extracts

	malt		hop		yeast biomass	
fatty acid	%	SD[b]	%	SD	%	SD
8:0	1.39	0.01	nd[c]	nd	0.51	0.01
10:0	0.14	0.00	6.45	0.04	3.60	0.12
12:0	nd	nd	13.58	0.10	1.24	0.08
14:0	0.20	0.00	1.10	0.01	1.80	0.00
16:0	19.10	0.13	10.64	0.03	23.06	0.13
16:1 (n-7)	0.21	0.01	0.49	0.01	23.44	0.01
18:0	1.70	0.01	1.48	0.00	8.70	0.02
18:1 (n-9)	12.57	0.09	2.97	0.01	19.62	0.09
18:2 (n-6)	57.08	0.07	23.91	0.05	6.29	0.07
18:3 (n-3)	6.85	0.03	23.58	0.05	1.17	0.03
20:0	nd	nd	nd	nd	0.34	0.01
20:1 (n-11)	0.34	0.01	nd	nd	nd	nd
20:4 (n-6)	0.82	0.02	5.49	0.01	nd	nd
22:0	0.26	0.03	nd	nd	nd	nd
22:1 (n-9)	0.40	0.03	nd	nd	nd	nd
22:2 (n-13)	nd	nd	1.92	0.02	15.96	0.03
24:0	nd	nd	2.88	0.01	nd	nd
24:1 (n-9)	nd	nd	4.01	0.02	nd	nd

[a] Mean of three replications. [b] SD, standard deviation. [c] nd, not detectable.

Table 2. Total Lipid Extract in Wort Samples for Mash A

	decoction mash A[a]		
total lipids	sweet wort (n = 8)	bitter wort (n = 8)	pitched wort (n = 4)
mg/L	134.00 a	47.00 b	124.00 a
SD[b]	33.00	7.00	17.00

[a] Different letters indicate statistical differences (P < 0.001) between samples. [b] SD, standard deviation

Table 3. Total Lipid Extract in Wort Samples for Mash B

	infusion mash B[a]		
total lipids	sweet wort (n = 5)	bitter wort (n = 6)	pitched wort (n = 2)
mg/L	84.00 a	46.00 b	160.00 c
SD[b]	9.00	10. 00	16.00

[a] Different letters indicate statistical differences (P < 0.001) between samples. [b] SD, standard deviation

Table 4. Fatty Acid Composition of Lipid Extract in Wort Samples for Mash A

	decoction mash A[a]					
	sweet wort		bitter wort		pitched wort	
fatty acid	%	SD[b]	%	SD	%	SD
8:0	9.56 a	0.46	4.94 b	0.12	0.20 c	0.00
10:0	5.77 a	0.42	5.83 a	0.18	1.27 b	0.01
12:0	0.56 a	0.05	0.63 a	0.03	0.52 b	0.01
14:0	1.17 a	0.06	0.98 a	0.58	0.81 a	0.01
16:0	16.18 a	0.16	17.37 b	0.35	15.66 c	0.11
16:1 (n-7)	0.68 a	0.02	0.79 b	0.03	11.78 c	0.10
18:0	3.01 a	0.01	2.12 b	0.02	8.24 c	0.00
18:1 (n-9)	16.71 a	0.13	14.66 b	0.20	16.24 c	0.01
18:2 (n-6)	23.47 a	0.11	25.31 b	0.44	10.35 c	0.01
18:3 (n-3)	20.66 a	0.12	25.38 b	0.39	12.53 c	0.02
22:2 (n-13)	1.41 a	0.21	1.34 a	0.19	21.40 b	0.27

[a] Mean of three replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation.

Table 5. Fatty Acid Composition of Lipid Extracts in Wort Samples for Mash B

	infusion mash B[a]					
	sweet wort		bitter wort		pitched wort	
fatty acid	%	SD[b]	%	SD	%	SD
8:0	16.16 a	1.40	12.14 b	0.42		0.05
10:0	14.94 a	1.65	11.61 b	0.30	4.29 c	0.21
12:0	0.46 a	0.07	0.35 a	0.01	1.87 b	0.07
14:0	0.55 a	0.03	0.50 a	0.01	1.64 b	0.04
16:0	13.45 a	0.17	13.80 a	0.10	20.58 b	0.20
16:1 (n-7)	0.96 a	0.12	0.83 a	0.03	16.21 b	0.13
18:0	2.00 a	0.08	2.14 a	0.02	5.32 b	0.06
18:1 (n-9)	9.13 a	0.38	9.88 a	0.10	10.96 b	0.47
18:2 (n-6)	29.50 a	1.14	31.83 a	0.40	4.84 b	0.04
18:3 (n-3)	10.96 a	1.65	14.84 b	0.36	1.07 c	0.07
22:2 (n-13)	1.26 a	0.21	0.93 a	0.02	32.33 b	0.52

[a] Mean of three replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation

The values of total lipids of bitter wort for mashes A and B were similar; the hot break and its separation during boiling and wort clarification allowed the removal of lipids in both infusion and decoction systems.

The total lipids content went up again in the pitched wort, for both the infusion and decoction system mash. This behavior can be explained considering the contribution given in these phases by both lipid constituents of yeast cells and lipids biosynthesized by yeast metabolism. As reported in the current literature, the mechanisms of fatty acid and lipid synthesis are well-known in yeast cells, which are capable of growing on carbohydrate-containing media and accumulating significant amounts of intracellular and membrane lipids (e.g., triacylglycerols and sterols). In addition, some yeasts can produce extracellular lipids (or glycolipids) under oxygenated culture conditions (*6*). The fatty acid composition of lipid extracts of wort samples is reported in **Table 4** for the decoction mash and in **Table 5** for the infusion mash.

For the decoction mash, linoleic acid was the major fatty acid found in the lipid extract from sweet wort (23.47%), followed by linolenic (20.66%), oleic (16.71%), palmitic (16.18%), caprylic (9.56%), capric (5.77%), and stearic (3.01%) acids, respectively.

The fatty acids profile of lipids in the bitter wort was found to be similar to that observed for sweet wort lipids. The dominant fatty acids were linolenic and linoleic acids (25.38 and 25.31%), whereas docosadienoic acid was the prevailing fatty acid (21.40%) in lipid extract from pitched wort. The others found

Table 6. Total Lipid Extract in Beer Samples for Mash A

	decoction mash A[a]		
total lipids	green beer	matured beer	filtered beer
mg/L	90.00 a	88.00 a	67.00 b
SD[b]	11.00	14.00	5.00

[a] Mean of four replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation

Table 7. Total Lipid Extract in Beer Samples for Mash B

	Infusion mash B[a]		
total lipids	green beer	matured beer	filtered beer
mg/L	85.00 a	82.00 a	33.00 b
SD[b]	10.00	9.00	8.00

[a] Mean of three replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation

fatty acids were oleic (16.24%), palmitic (15.66%), linolenic (12.53%), palmitoleic (11.78%), linoleic (10.35%), and stearic (8.24%) acids.

The different compositions among fatty acids of pitched, sweet, and bitter worts could be justified considering the presence of yeast biomass and the lipids in pitched wort.

For an infusion mash, linoleic acid was the major fatty acid (29.50%) found in the lipid extract from sweet wort, followed by caprylic (16.16%), capric (14.94%), palmitic (13.45%), linolenic (10.96%), and oleic (9.13%) acids, respectively.

Linoleic and linolenic acids were the dominant fatty acids (31.83 and 14.84%) observed in bitter wort, followed by palmitic (13.80%), caprylic (12.14%), capric (11.61%), and oleic (9.88%) acids, respectively. For the infusion mashing, the fatty acids profile of lipids found in bitter wort was similar to that observed in sweet wort.

Finally, docosadienoic acid was the dominant fatty acid (32.33%) in lipid extract from pitched wort, followed by palmitic (20.58%), palmitoleic (16.21%), oleic (10.96%), stearic (5.32%), and linoleic (4.84%) acids, respectively. The composition of the lipid extract of sweet and bitter wort was found to be essentially unchanged. The addition of yeast starter culture of *S. cerevisiae* (Saflager W-34/70) in pitched wort caused a modification of fatty acid profile. Conventionally, fatty acids available for yeast catabolism include those derived from (i) microsomal alkane oxidation and (ii) extracellular hydrolysis of lipids and (iii) those supplied exogenously in the growth medium (e.g., brewing raw materials, wort, green beer, matured and filtered beer). With regard to fatty acids derived from lipid hydrolysis, several yeasts are known to secrete inducible lipases to degrade TAG-containing substrates to glycerol and fatty acids. Pathways of fatty acid catabolism and anabolism and their regulation have been studied extensively in yeasts (*6*, *16*, *17*). This series of reactions leads to the synthesis of long-chain fatty acids in yeast cells and takes place in a multienzyme complex (labeled as fatty acid synthase). The unsaturated fatty acids, which play important roles in yeast physiology (e.g., membrane integrity) and biotechnology (e.g., ethanol tolerance), include palmitoleic and oleic acids. Usually, the sum of both compounds constitutes > 50% of both fatty acids in *S. cerevisiae* cell membranes. The others consist mainly of the saturated fatty acids, primarily palmitic and lesser amounts of myristic and stearic acids (*6*, *7*, *17*). On the basis of these considerations, the results reported in **Tables 6** and **7** are consistent with the hypothesis that the addition of starter culture of *S. cerevisiae* in pitched wort causes a modification of the fatty acids profile.

Table 8. Fatty Acid Composition of Lipid Extracts in Beer Samples for Mash A

	decoction mash A[a]					
	green beer		matured beer		filtered beer	
fatty acid	%	SD[b]	%	SD	%	SD
8:0	0.15 a	0.00	0.07 b	0.00		
10:0	0.63 a	0.01	0.33 b	0.00		
12:0	0.26 a	0.00	0.10 b	0.00		
14:0	0.55 a	0.01	0.23 b	0.00		
16:0	18.03 a	0.25	9.81 b	0.06	14.39 c	0.14
16:1 (n-7)	12.19 a	0.14	4.92 b	0.03		
18:0	13.94 a	0.17	7.60 b	0.02	6.19 c	0.08
18:1 (n-9)	31.04 a	0.57	34.26 b	0.07	55.70 c	0.60
18:2 (n-6)	14.62 a	0.34	13.22 b	0.03	11.89 c	0.86
18:3 (n-3)	2.20 a	0.12	3.47 b	0.06		0.10
22:2 (n-13)	6.38 a	0.09	25.40 b	0.05	11.74 c	

[a] Mean of three replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation.

Table 9. Fatty Acid Composition of Lipid Extracts in Beer Samples for Mash B

	infusion mash B[a]					
	green beer		matured beer		filtered beer	
fatty acid	%	SD[b]	%	SD	%	SD
8:0	2.81 a	0.17	5.62 b	0.02	0.78 c	0.05
10:0	2.49 a	0.08	9.42 b	0.22	2.88 c	0.17
12:0	0.24 a	0.01	1.42 b	0.10	27.42 c	0.51
14:0	0.34 a	0.00	1.94 b	0.08	0.94 c	0.05
16:0	18.54 a	0.03	17.28 b	0.63	11.92 c	0.40
16:1 (n-7)	0.30 a	0.00	10.56 b	0.08	2.38 c	0.38
18:0	22.70 a	0.02	4.43 b	0.03	4.44 b	0.20
18:1 (n-9)	48.43 a	0.17	24.39 b	0.91	36.72 c	0.30
18:2 (n-6)	0.63 a	0.00	6.02 b	0.06	2.76 c	0.06
18:3 (n-3)	1.11 a	0.01	3.12 b	0.03	3.35 b	0.16
22:2 (n-13)	1.41 a	0.00	15.45 b	1.21	5.60 c	0.45

[a] Mean of three replications. Different letters indicate statistical differences ($P < 0.001$) between samples. [b] SD, standard deviation.

Beer Lipids. Table 6 reports the lipid extracts of beer samples (green beer, matured beer, and filtered beer) for decoction mash A.

Lipid content decreased during the brewing process from pitched wort to filtered beer in both decoction and infusion mashing systems. A large part of lipids in pitched wort was introduced by the addition of yeast biomass to the wort. The yeast metabolism and the separation of yeast biomass at the end fermentation process resulted in a consistent decrease of the lipid content of green beer. The lipid content held steady during the maturation step and decreased slightly during beer filtration. The filtration allowed the separation of some lipids; the maturation of beer did not have an effect on the lipid content for the mash A.

Table 7 reports the results for lipid extraction of beer samples for infusion mash B.

For mash B, the lipid contents of green beer and matured beer were similar to those of the decoction mash, whereas a consistent decrease of lipid content was observed during filtration (67.18± 5.68 mg/L for decoction mashing, 32.70±8.38 mg/L for infusion mashing). The matured beer obtained with mash B was filtered with a bell-shaped kieselguhr filter (the kieselguhr filter is a type of filter consisting of a thick layer of kieselguhr through which the beer is pumped) having a smaller pore size than the kieselguhr filter used for mash A; therefore, the filtration was more effective and the lipid fraction more retained by the filter. The filtered beer B exhibited almost half the lipid content of filtered beer A. Therefore, it is possible to conclude that filtration is an important technological step affecting the final lipid content of final beer.

The fatty acid composition of lipid extracts of beer samples is reported in **Table 8** for the decoction mash and in **Table 9** for the infusion mash.

For the decoction mash, oleic acid was the dominant fatty acid in the lipid extract from green beer (31.04%), followed by palmitic (18.03%), linoleic (14.62%), stearic (13.94%), palmitoleic (12.19%), and docosadienoic (6.38%) acids. Metabolically active yeast cells dispersed in the beer, as well as raw materials and mashing, also affected the fatty acid profile of lipids in green beer. During maturation, the finished beer acquires a smooth, mellow flavor; in the final phases yeast cells flocculate, thus allowing the final removal. Therefore, the fatty acid composition of matured beer is directly influenced by the process of maturation. In particular, we can observe an increase of oleic and docosadienoic acid contents and, at the same time, a decrease of palmitic and palmitoleic acid contents. In the filtered beer the removal of both yeast biomass and trub resulted in a variation of fatty acid composition of residual lipids. For decoction mash, oleic acid was the main fatty acid found in the lipid extract from filtered beer (55.7%), followed by palmitic (14.39%), linoleic (11.89%), linolenic (11.74%), and stearic (6.19%) acid.

For the infusion mash, oleic acid was the dominant fatty acid in the lipid extract from green beer (48.43%), followed by stearic (22.7%) and palmitic acid (18.54%). During the maturation, yeast growth determines an increased amount of biosynthesized fatty acids. Accordingly, in the matured beer a higher percentage of docosadienoic acid (15.45%), palmitoleic (10.56%), and linoleic (6.02%) acids (probably produced by yeasts) was observed. The filtration caused a decrease of docosadienoic (5.6%), palmitic (11.92%), and linoleic (2.76%) acid. In the filtered beer the dominant fatty acid was oleic acid (36.72%), followed by lauric (27.42%) and palmitic acid (11.92%).

Figure 2 reports the time course of the lipid fraction in the brewing process. The data are referred to the effective volumes of wort and beer samples: for mash A, 147 L of sweet wort, 116 L of bitter wort, 116 L of pitched wort, 116 L of green beer, 116 L of matured beer, and 86 L of filtered beer; for mash B, 142 L of sweet wort, 118 L of bitter wort, 118 L of pitched wort, 118 L of green beer, 118 L of matured beer, and 88 L of filtered beer.

For both decoction and infusion systems the lipid fraction decreased from sweet to bitter wort. The phase of the brewing process that transforms sweet wort into bitter wort consists essentially of three technological steps: (i) wort boiling, (ii) hopping, and (iii) clarification in a whirlpool. Due to the presence of fermenting yeast cells, the lipid content rose again in pitched wort. Both fermentation and yeast biomass removal decreased the lipid content of green beer, whereas that of matured beer remained unchanged. Beer filtration allowed the decreasing of lipid content in filtered beer; such decrease was more evident in mash B than in mash A because of the proper bell-shaped kieselguhr filter used and smaller size of its pore.

The monitoring of lipid content and composition during the beer production process highlights the importance of some technological operations as important steps to reduce the lipid content in bitter wort. Accordingly, a few guidelines should be hypothesized. Among them, boiling and clarification of sweet wort in whirlpool seems to influence the lipid content and composition in raw materials, wort, and beers. Besides, the use of infusion mashing system should be emphasized as a tool for obtaining sweet wort characterized by lower lipid content than the use of a decoction system. This result could be justified considering that the infusion system involves the intimate mixing of the ground material with hot water and avoids the contact of the mash with boiling water, because all phases of mashing are

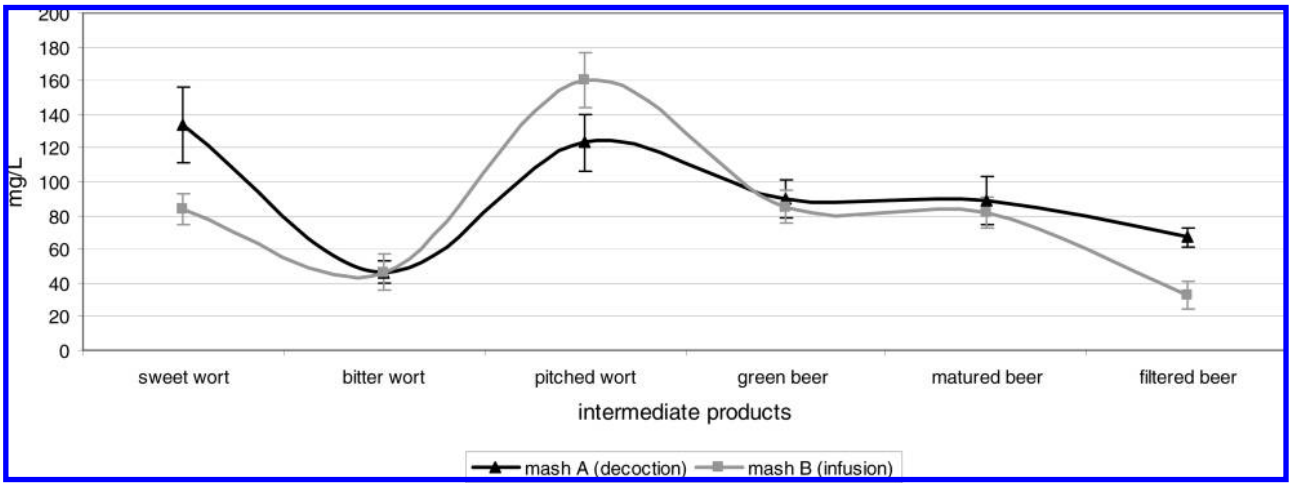

Figure 2. Trend of lipid fraction in brewing process for mashes A and B.

developed in the mash tun. On the other hand, the decoction system initially forecasts the finer grinding of malt and its mixing with lower temperature water. Finally, portions of the untreated mash are separately boiled and later mixed with the treated mash tun: this step determines a gradual increase of the temperature of the entire mash. Under these conditions, extensive proteolysis and solubilization occur.

The presence of metabolically active yeast cells is another factor having a great influence on fatty acid composition of lipids found in both brewing byproduct and finished beer. The presence of yeast cells may be considered a critical point, which is clearly strongly influenced by the filter pore size. Passage through the kieselguhr filter reduced the levels of fats in finished beer. The different type of filter used in the infusion mashing process was found to be more efficient in removing lipids. Therefore, filtration can be considered an additional technological step affecting the final lipid content of the final beer.

ABBREVIATIONS USED

HRGC-FID, high-resolution gas chromatography−flame ionization detector; TAGs, triacylglycerols; DAGs, diacylglycerols; MAGs, monoacylglycerols; FFAs, free fatty acids; PLs, polar lipids; FAMEs, fatty acid methyl esters.

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