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Influence of Lipids in the Generation of Phenylacetaldehyde in Wort-Related Model Systems

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The effect of lipids on the formation of the Strecker aldehyde phenylacetaldehyde during wort boiling was studied to determine the role that small changes in the lipid content of the wort have in the production of significant flavor compounds in beer. Wort was treated with 0−2.77 mmol per liter of glucose, linoleic acid, or 2,4-decadienal and heated at 60−98 °C for 1 h. After this time, the amount of the Strecker aldehyde phenylacetaldehyde increased in the samples treated with linoleic acid or decadienal but not in the samples treated with glucose. Thus, the amount of phenylacetaldehyde produced in the presence of linoleic acid was 1.1−2.5 times the amount of the Strecker aldehyde produced in the control wort, and this amount increased to 3.6−4.6 times when decadienal was employed. The higher reactivity of decadienal than linoleic acid for this reaction decreased with temperature and was related to the oxidation of linoleic acid that occurred to a higher extent at higher temperatures. The above results suggest that lipids can contribute to the formation of Strecker aldehydes during wort boiling and that changes in the lipid content of the wort will produce significant changes in the formation of Strecker aldehydes in addition to other well-known consequences in beer quality and yeast metabolism. On the other hand, because of the high glucose content in wort, small changes in its content are not expected to affect the amount of Strecker aldehydes produced.

KEYWORDS: Beer quality; carbonyl−amine reactions; lipid oxidation; Maillard reaction; Strecker aldehydes; wort boiling

INTRODUCTION

One of the most important issues in modern brewing is how to produce beer having adequate flavor and foam stability. In this context, the presence of lipids has long been considered to have adverse effects on beer quality. For example, excessive amounts of unsaturated fatty acids in wort inhibit the synthesis of ester components (*1, 2*), and oxidized derivatives of linoleic acid can give beer a stale flavor and damage the flavor stability (*3, 4*). Furthermore, lipids are antifoaming agents and are able to decrease the foam forming, foam lacing, and foam stability of beer (*5*). On the other hand, long-chain unsaturated fatty acids present in lipid-rich worts have a significant influence on fermentation. Lipids supplement the oxygen demand of yeast cells, favor the sterol biosynthesis, and lead to a more intensive and significantly faster fermentation (*6, 7*).

Most lipids involved in the brewing process are derived from malt, and they occur in various forms including simple lipids (fatty acids, triglycerides, and other neutral lipids), complex lipids (glycolipids and phospholipids), and bound lipids such as those bound with starch grains. However, not all of the lipids have an adverse effect, and the balance among these forms of lipids subtly affects the beer quality and the efficiency of the beer-brewing process. In fact, neither the most appropriate lipid balance nor the preferable total lipid content in wort is completely understood at present. For example, despite the widely accepted preference among brewers for a lauter wort as clear as possible, Kühbeck et al. found that fermentation performance may be improved by employing worts with a higher content in linoleic acid with not significant losses in the final quality of the beer (*8*).

In an attempt to understand the changes produced in beer as a consequence of changes in the lipid content of the employed wort, the objective of this study was to determine the effect that increasing amounts of lipids have in the formation of Strecker aldehydes during wort boiling. Due to the high temperatures during the boiling of wort, flavor components are not only expelled but also recreated (*9*). Among these recreated compounds, Strecker aldehydes are produced as a consequence of Maillard reaction (*10*). However, lipid oxidation also occurs during wort boiling (*11*), and recent studies have suggested that secondary and tertiary lipid oxidation products are also able to

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degrade amino acids to their corresponding Strecker aldehydes (*12*−*14*). Therefore, an increase in the amount of lipids in wort should produce an increase in the formation of Strecker aldehydes during wort boiling. This study analyzes the changes produced in the Strecker aldehyde phenylacetaldehyde, which is a primary odorant in beer (*15, 16*). In addition, model reactions involving phenylalanine, unoxidized lipids, and carbohydrates were also assayed in an attempt to understand some of the reaction pathways involved in complex mixtures in which amino acids, lipids, and carbohydrates are present.

MATERIALS AND METHODS

Materials. 2,4-Decadienal, fructose, glucose, hexanal, linoleic acid, linoleic acid methyl ester, phenylacetaldehyde, L-phenylalanine, and ribose were purchased from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), Fluka (Buschs, Switzerland), or Merck (Darmstadt, Germany). All other chemicals were purchased from reliable commercial sources.

The wort was prepared by mashing at 55 °C with 86 kg of pilsner malt (Dingemans, Stabroek, Belgium) in 220 L of water for 10 min. The pH was adjusted to 5.5 with lactic acid (food grade) and heated first to 63 °C for a 45 min β-amylase rest, then to 72 °C for 20 min (α-amylase) until saccharification (iodine test was used to make sure that all starch was hydrolyzed), and, finally, at 78 °C to denature the enzymes. After filtration in the lauter tun and subsequent sparging with water at 78 °C, 530 L of clear wort in the boiling kettle was obtained. This wort was treated with hop extract (Styrian Goldings) (40% α-acids) to obtain a final bitterness of 20 EBU, and the pH was adjusted to 5.2 with lactic acid. After 1 h of boiling with 6% evaporation (the final quantity of wort was 500 L), the wort was clarified by transferring it to the whirlpool (20 min rest) and stored in small plastic containers until use (−18 °C).

The lipid content of the prepared wort was determined by extraction with chloroform/methanol (2:1). It was 1.23 g/L. However, the hydrolysis of this lipid extract with sodium methoxide and its later esterification with methanol in acid media showed negligible amounts of methyl fatty esters, therefore indicating that the prepared wort had only trace amounts of fatty acid derivatives susceptible to produce oxidized derivatives upon heating.

Formation of Phenylacetaldehyde in Phenylalanine/Lipid/Carbohydrate Binary and Tertiary Reaction Mixtures. Mixtures of 25 μmol (50 μmol in tertiary mixtures) of phenylalanine and 25 μmol of the lipid and/or 25 μmol of the carbohydrate in 0.5 mL of 0.3 M sodium citrate, pH 3, were introduced in Schott Duran test tubes (16 × 1.5 cm), which were closed and heated at 60−100 °C for 1 h. At the end of the heating period, samples were cooled, diluted with 1 mL of acetonitrile and 50 μL of internal standard solution (54.8 mg of methyl heptanoate in 25 mL of methanol), and analyzed by GC-MS.

GC-MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (mass selective detector-quadrupole type). A fused-silica HP5-MS capillary column (30 m × 0.25 mm i.d.; coating thickness, 0.25 μm) was used. Working conditions were as follows: carried gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature, programmed from 70 (1 min) to 240 at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD, 280 °C; and ionization EI, 70 eV.

Quantification of phenylacetaldehyde was carried out by preparing standard curves of the aldehyde in the 1.55 mL of solution prepared for GC-MS injection. For each curve, eight different concentration levels of the aldehyde were used. Phenylacetaldehyde content was directly proportional to the aldehyde/internal area ratio ($r > 0.99$, $p < 0.0001$). The coefficients of variation were lower than 10%.

When ternary mixtures were studied, a synergism factor (S_F) was calculated as the ratio between the experimental phenylacetaldehyde amount produced by the ternary mixture and the theoretical amount of phenylacetaldehyde that should have been produced considering the amount of phenylacetaldehyde formed in the binary mixtures of the corresponding lipids or carbohydrates with phenylalanine. A $S_F > 1$ indicated a positive synergism. A $S_F < 1$ indicated a negative synergism.

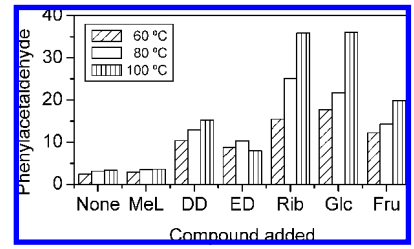

Figure 1. Formation of phenylacetaldehyde in binary mixtures of phenylalanine with either lipids or carbohydrates heated for 1 h at 60, 80, or 100 °C. Values are given in micromoles of phenylacetaldehyde per 100 μmol of phenylalanine. Abbreviations: MeL, methyl linoleate; DD, 2,4-decadienal; ED, 4,5-epoxy-2-decenal; Rib, ribose; Glc, glucose; Fru, fructose.

Formation of Phenylacetaldehyde in Wort Heated in the Presence of Lipids or Carbohydrates. Wort (11 mL) was heated in 25 mL Pyrex test tubes for 0−90 min at 60−98 °C in the presence of 0−30.52 μmol of glucose, linoleic acid, or 2,4-decadienal. These amounts were selected because they were close to the lipid content in older kettle-full worts (*17*). After heating, samples were cooled, and the contents of phenylacetaldehyde and hexanal were determined by GC-MS after solid-phase microextraction (SPME).

SPME was carried out in a 20 mL headspace vial filled with 5 mL of heated wort, together with 1.75 g of NaCl and 50 μL of a 2-heptanol solution (10 mg in 50 mL of ethanol), which was used as internal standard. The vial was placed in the tray of the CombiPAL (CTC Analytics, Zwingen, Switzerland), where it was preincubated at 40 °C for 5 min. A carboxen/polydimethylsiloxane/divinylbenzene fiber (CAR/PDMS/DVB, Supelco, Bellefonte, PA) was used for the extraction of volatiles in the headspace of the vial during 15 min at 40 °C.

Volatiles were desorbed for 2 min at 250 °C in the split/splitless injector of a Trace GC Ultra (Thermo, Waltham, MA) working in the split mode (split ratio equal to 8). An RTX-5 MS column (60 m × 0.25 mm i.d.; coating thickness, 0.5 μm; Restek, Bellefonte, PA) was used. A constant flow of 1.5 mL helium per minute was applied. Oven conditions were as follows: from 45 (3 min) to 270 °C (2 min) at 12 °C/min. After separation, the volatiles were analyzed with a dual-stage quadrupole (DSQ) MS (Thermo), which was set to detect ions with a mass to charge ratio (m/z) of 33−260 in the electron impact mode. The data were analyzed using Xcalibur software (Thermo).

Quantification of phenylacetaldehyde and hexanal was carried out by preparing standard curves of the aldehydes in the unheated wort and extracting the volatile using the above-described conditions. For each curve, six different concentration levels of the aldehydes were used. Phenylacetaldehyde and hexanal contents were directly proportional to the aldehyde/internal area ratio ($r > 0.99$, $p < 0.0001$). The coefficients of variation in the wort heated in the presence of lipids or carbohydrates were lower than 20%.

RESULTS

Formation of Phenylacetaldehyde in Binary Mixtures of Phenylalanine with either Lipids or Carbohydrates. When mixtures of phenylalanine with either lipids or carbohydrates were heated for 1 h at 60−100 °C, the formation of phenylacetaldehyde was observed (**Figure 1**). The amount of Strecker aldehyde produced depended on the lipid or carbohydrate employed and the reaction temperature. Thus, the unoxidized lipid assayed was the least reactive compound for this reaction. However, the amount of phenylacetaldehyde produced in the presence of the lipid at 60 °C was 21% higher than the phenylacetaldehyde produced in the absence the lipid. In addition, the amount of Strecker aldehyde produced increased with the temperature of the reaction.

When the assayed lipid was a secondary lipid oxidation product such as decadienal, the amount of phenylacetaldehyde produced was much higher than when the unoxidized lipid was tested, and this difference increased with the temperature. Thus, decadienal/phenylalanine mixtures produced 3.5 times more

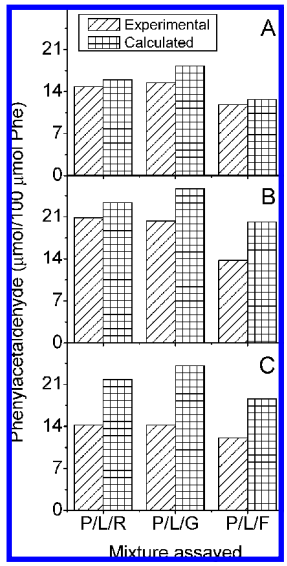

Figure 2. Formation of phenylacetaldehyde in ternary mixtures of phenylalanine, lipids, and carbohydrates heated for 1 h at 60 °C. Abbreviations: P/L/R, phenylalanine/lipid/ribose; P/L/G, phenylalanine/lipid/glucose; P/L/F, phenylalanine/lipid/fructose. The lipids assayed were (**A**) methyl linoleate, (**B**) 2,4-decadienal, and (**C**) 4,5-epoxy-2-decenal. The figure also includes the theoretical additive amount of phenylacetaldehyde that should have been produced considering the Strecker aldehyde formed in the corresponding binary mixtures of the amino acid with either the lipid or the carbohydrate.

phenylacetaldehyde than methyl linoleate/phenylalanine mixtures at 60 °C, and this ratio increased to 4.2 at 100 °C.

The assayed tertiary lipid oxidation product 4,5-epoxy-2-decenal also produced the Strecker degradation of the amino acid to a high extent, although epoxydecenal/phenylalanine mixtures produced slightly lower amounts of phenylacetaldehyde than decadienal/phenylalanine mixtures. Thus, epoxydecenal/phenylalanine mixtures produced 3.0 times more phenylacetaldehyde than methyl linoleate/phenylalanine mixtures at 60 °C, and this ratio decreased to 2.2 at 100 °C.

When the lipid was substituted by a carbohydrate, phenylacetaldehyde was also produced to a high extent. The less reactive of the carbohydrates assayed was fructose. Fructose/phenylalanine mixtures produced 12−20 μmol of phenylacetaldehyde/100 μmol of phenylalanine, which was similar to the 10−15 μmol of phenylacetaldehyde/100 μmol of phenylalanine produced by decadienal/phenylalanine mixtures. However, glucose and ribose were more reactive for this reaction, and this reactivity increased with temperature. Thus, ribose/phenylalanine mixtures produced 1.5 times the phenylacetaldehyde formed in decadienal/phenylalanine mixtures at 60 °C, and this ratio increased to 2.3 at 100 °C. Analogous results were also found for glucose/phenylalanine mixtures.

Formation of Phenylacetaldehyde in Ternary Mixtures of Phenylalanine with Lipids and Carbohydrates. When phenylalanine was heated simultaneously with lipids and carbohydrates (**Figure 2**), the differences observed among the different lipids were greatly reduced as compared to the above-described binary mixtures. Thus, decadienal/carbohydrate/phenylalanine ternary mixtures produced only 1.2−1.4 times the phenylacetaldehyde produced by methyl linoleate/carbohydrate/phenylalanine mixtures. In addition, ternary mixtures of epoxydecenal, carbohydrate, and phenylalanine produced phenylacetaldehyde to a similar extent as methyl linoleate/carbohydrate/phenylalanine mixtures.

Table 1. Synergism Factors (S_F) Determined for the Formation of Phenylacetaldehyde in Lipid/Carbohydrate/Phenylalanine Reaction Mixtures[a]

lipid	carbohydrate	S_F
methyl linoleate	ribose	0.93
methyl linoleate	glucose	0.85
methyl linoleate	fructose	0.93
2,4-decadienal	ribose	0.89
2,4-decadienal	glucose	0.79
2,4-decadienal	fructose	0.68
4,5-epoxy-2-decenal	ribose	0.65
4,5-epoxy-2-decenal	glucose	0.59
4,5-epoxy-2-decenal	fructose	0.65

[a] S_F is defined as the ratio between the experimental phenylacetaldehyde amount produced by the ternary mixture and the theoretical amount of phenylacetaldehyde that should have been produced considering the amount of phenylacetaldehyde formed in the binary mixtures of the corresponding lipids or carbohydrates with phenylalanine.

These reduced differences among the different lipids tested were a consequence of the existence of a negative synergism between the lipids and the carbohydrates, which depended on the employed lipid. To calculate the synergism between lipids and carbohydrates, **Figure 2** also includes the theoretical additive amount of phenylacetaldehyde that should have been produced considering the amount of phenylacetaldehyde formed in the binary mixtures of the corresponding lipids or carbohydrates with phenylalanine (**Figure 1**). The ratio between the experimental value and the calculated value of phenylacetaldehyde is defined as S_F. **Table 1** collects the S_F values calculated for the different samples analyzed in this study. All determined S_F values were lower than 1, therefore indicating that a negative synergism was always produced when lipids and carbohydrates were heated simultaneously with phenylalanine. However, the S_F value mainly depended on the employed lipid. Thus, it was close to 1 (0.85−0.93) for methyl linoleate, lower for 2,4-decadienal (0.68−0.89), and much lower for epoxydecenal (0.59−0.65).

Formation of Phenylacetaldehyde in Wort Heated in the Presence of Lipids or Carbohydrates. The wort is a diluted aqueous solution of different compounds among which carbohydrates and amino acids are present. Therefore, its heating should produce the Strecker degradation of the amino acids, among other reactions, as a consequence of the Maillard reaction produced. **Figure 3** shows that, when wort was heated in the absence of added compounds, the Strecker aldehyde phenylacetaldehyde was produced and the amount of this aldehyde increased as a function of the temperature of the reaction. However, the amount of phenylacetaldehyde produced depended also on the addition of small amounts of other compounds. Because these compounds were added to a much lower extent than the carbohydrate content present in the wort [the glucose content in wort is 50−60 mmol/L (*18*) and the tested compounds were added in the range 0−2.77 mmol/L], the addition of small amounts of glucose did not influence the amount of phenylacetaldehyde produced as compared to control (**Figure 3A**). However, when the compound added was an unoxidized lipid (linoleic acid) or the secondary lipid oxidation product decadienal, significant increases in the amount of phenylacetaldehyde were observed (**Figure 3B**,**C**, respectively). Thus, the addition of 0−2.77 mmol of linoleic acid per liter of wort increased the amount of phenylacetaldehyde produced 1.1−2.5 times depending on the reaction temperature (the higher temperature, the higher the increase in phenylacetaldehyde produced). As expected, according to the above-described results obtained in model systems, this effect was higher when decadienal was

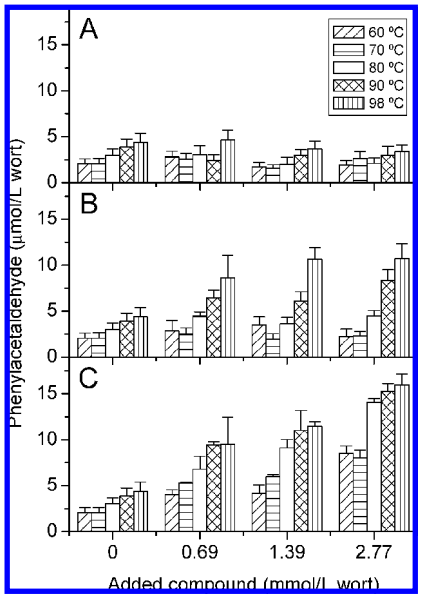

Figure 3. Formation of phenylacetaldehyde in wort heated for 1 h at 60−98 °C in the presence of (**A**) glucose, (**B**) linoleic acid, and (**C**) 2,4-decadienal.

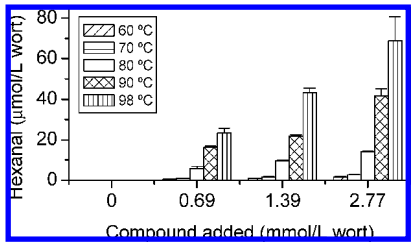

Figure 4. Formation of hexanal in wort heated for 1 h at 60−98 °C in the presence of linoleic acid.

added, and the amount of phenylacetaldehyde produced increased 3.6−4.6 times as compared to control. This difference in the reactivity between decadienal and linoleic acid decreased as a function of the reaction temperature. Thus, decadienal produced 3.8 times the amount of Strecker aldehyde formed by linoleic acid at 60 °C, and this ratio decreased to 1.5 times at 98 °C. This was a consequence of the need of the unoxidized fatty acid to be oxidized as a preliminary step to the amino acid degradation, and this oxidation should take place to a higher extent at 98 °C than at 60 °C.

A confirmation of this increased oxidation at higher temperatures was obtained by determination of the hexanal formed during wort heating in the presence of linoleic acid at the several temperatures assayed (**Figure 4**). As expected from the very low lipid content of the employed wort, hexanal was not produced when the fatty acid was not added. However, small additions of linoleic acid induced the formation of hexanal, and the amount of this lipid oxidation product increased with the temperature. Thus, very low oxidation was produced at either 60 or 70 °C, which was in agreement with the insignificant contribution of linoleic acid to the production of phenylacetaldehyde in wort at these temperatures (**Figure 3**). However, higher temperatures induced the oxidation of the lipid, and this was parallel to the increase of phenylacetaldehyde produced in wort at these temperatures. In fact, lipid oxidation, measured as the formation of hexanal, and phenylacetaldehyde formation were correlated ($r = 0.94$, $p < 0.0001$).

In addition to the amount of the added lipid, the time of wort heating also determined the amount of phenylacetaldehyde produced. **Figure 5** shows the amount of phenylacetaldehyde produced in wort after the addition of 0.69 mmol of either glucose or linoleic acid per liter of wort as a function of the heating time at three temperatures: 60, 80, and 98 °C. As expected, according to the above-described results, the addition of glucose had a small effect in the formation of phenylacetaldehyde (only when the wort was heated at 98 °C for a long heating period did the amount of phenylacetaldehyde produced seem to be higher than control). On the contrary, the addition of linoleic acid increased the formation of phenylacetaldehyde at both 80 and 98 °C, and this increase seemed to be linear as a function of the heating time ($r > 0.94$, $p < 0.06$). In addition, this increase was parallel to the lipid oxidation produced in the wort (**Figure 6**), which was also linear as a function on the heating time ($r > 0.982$, $p < 0.018$). Furthermore, lipid oxidation and phenylacetaldehyde formation were correlated ($r = 0.96$, $p < 0.0001$).

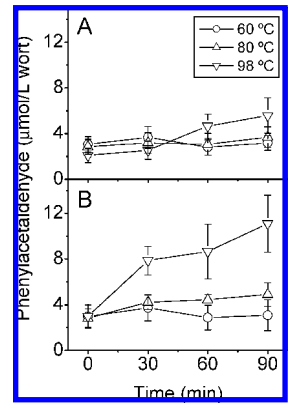

Figure 5. Formation of phenylacetaldehyde in wort heated in the presence of 0.69 mmol of (**A**) glucose and (**B**) linoleic acid per liter of wort.

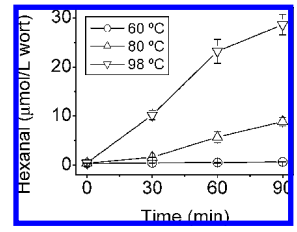

Figure 6. Formation of hexanal in wort heated in the presence of 0.69 mmol of linoleic acid per liter of wort.

DISCUSSION

The importance of fatty acids in wort has been known for a long time because they affect several beer qualities and yeast metabolism. Thus, wort lipids including fatty acids are necessary for the activation of yeast cell growth and significantly affect the fermentation process (*19*). In addition, some fatty acids have a high flavor-generating potential. Especially, linoleic and linolenic acids have received a great attention because their oxidative degradation may lead to the formation of a characteristic aging flavor (*20*−*22*).

The results obtained in the present study have pointed out a new role of lipids in wort, that is, their contribution to the Strecker degradation of amino acids. This contribution is produced not only by lipid oxidation products, as previously shown in model systems (*12*−*14*), but also by unoxidized lipids. Unoxidized lipids were oxidized during wort boiling, and the oxidation products formed degraded the amino acids. This oxidation, and the later degradation of the amino acids, was produced to different extents in binary mixtures of lipids and amino acids or when both types of compounds were heated in the presence of other food compo-

nents such as carbohydrates. In fact, when an unoxidized lipid and an amino acid were heated together, the amount of Strecker aldehyde produced was very limited. However, in the presence of carbohydrates, the lipid oxidation was promoted and mixtures of unoxidized lipids, carbohydrates, and amino acids produced Strecker aldehydes to similar extents as mixtures of oxidized lipids, carbohydrates, and amino acids.

The different behaviors of lipids for this reaction in the presence or in the absence of carbohydrates are likely another consequence of the described influence of Maillard reaction in the reaction rate and the compounds produced in the lipid oxidation process (*23*). In fact, Maillard reaction products have been found to promote lipid oxidation (*24*), therefore converting lipids into a much more suitable form to produce the Strecker degradation of the amino acids. This promoting effect of Maillard reaction for the oxidation of unsaturated lipids contrasts with the negative synergism observed for oxidized lipids and carbohydrates for this reaction, therefore suggesting that either the oxidized lipids or the products of reaction between oxidized lipids and amino acids are also modifying the Maillard reaction produced between the carbohydrates and the amino acid. The chemical nature of these complex reactions remains yet to be fully understood.

Previous studies have indicated secondary short- and long-chain oxodienes and tertiary lipid oxidation products having two oxygenated functions as being responsible for the Strecker degradation of amino acids (*12*−*14*). However, the high amounts of phenylacetaldehyde produced in methyl linoleate/carbohydrate/phenylalanine mixtures in comparison with tertiary mixtures containing either decadienal or epoxydecenal suggest that other lipid oxidation products might also be contributing to the Strecker degradation of amino acids and that study of the reactivity of other lipid oxidation products for this reaction is needed.

The above results suggest that lipids can contribute to the formation of Strecker aldehydes during wort boiling and that changes in the lipid content of the wort are going to produce significant changes in the formation of Strecker aldehydes. On the other hand, similar changes in the content of glucose are not expected to affect the amount of Strecker aldehydes produced because of the high content of glucose existing in wort.

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