- 2) La contribution de la CAT à réduire le phénomène de détérioration oxydative, plus précisément à combattre la peroxydation lipidique, devrait se limiter aux étapes de maltage et d'empâtage, compte tenu de la thermosensibilité de ses isoenzymes et en accord avec (**2, 8**).
- 3) Nous avons mis en évidence la présence dans l'orge et le malt de cinq formes de POD, quatre formes cationiques majoritaires et une fraction neutre / anionique minoritaire, qui catalysent la peroxydation de nombreux substrats phénoliques et de façon préférentielle celle de l'acide férulique, un dérivé hydroxycinnamique endogène de l'orge (**13**).
- 4) Les différentes fractions POD présentent des différences de stabilité thermique au cours du maltage, les fractions thermorésistantes étant les plus intéressantes sur le plan technologique.

En empêchant l'accumulation d'H_2O_2 pendant le brassage, les POD pourront ralentir la formation d'espèces réactives et protégeront le moût contre les réactions d'oxydation non spécifiques, notamment celle des acides gras polyinsaturés.
- 6) De par leur spécificité envers l'acide férulique (et *p*-coumarique), elles pourraient favoriser des réticulations intramoléculaires, pentosane-pentosane, protéine-protéine et / ou pentosane-protéine (**14**) et entraîner des problèmes de filtration au cours du brassage du moût.
- 7) La libération des noyaux hématiniques par suite de la dénaturation thermique de certaines formes enzymatiques de CAT et de POD pourra favoriser l'oxydation des acides gras polyinsaturés, en jouant le rôle de catalyseurs chimiques, en accord avec (**1, 12**).
- 8) La méthode proposée permet de sélectionner des variétés d'orge en fonction de leur potentiel oxydatif. On peut conclure que l'intervention des POD et CAT sur les propriétés sensorielles de la bière dépend du contexte physico-chimique du moût (pH, force ionique, humidité relative) et du traitement thermique appliqué pendant le touraillage.

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A global reduction power test instead of monitoring malt lipoxygenasic activity

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Descriptors
Enzymic activity, lipoxygenase, malt analysis, reducing agent, taste stability

SUMMARY
From recent works devoted to beer staling, reduction power emerged as the main parameter to monitor through mashing and boiling. Malt lipoxygenasic activity is not the only factor which governs wort oxidability. A more suitable measure for predicting flavour stability, based on the oxidation of linoleic acid in aqueous solution induced by a free radical initiator, has been developed. The antioxidative efficiency is given by the inhibition time in comparison with a control. This rapid and reproducible method enables to compare the efficiency of various antioxydants in the kettle. Correlations with beer ageing and comparisons with other tests are discussed.

Ein pauschaler Test der Reduktionskraft statt Überprüfung der Lipoxygenasenaktivität im Malz

Deskriptoren
Enzymaktivität, Geschmacksstabilität, Lipoxygenase, Malzanalyse, Reduktionsmittel

ZUSAMMENFASSUNG
Neuere Arbeiten im Bereich der Bieralterung zeigen, daß die Reduktionskraft als Hauptfaktor während des Maischens und Würzekochens eingesetzt werden kann. Die Malz-Lipoxygenasenaktivität ist nicht der einzige Faktor, der die Oxidierbarkeit der Würze beeinflußt. Ein geeigneteres Verfahren zur Vorhersage der Geschmacksstabilität wurde entwickelt, das auf der Oxidation von Linolensäure in wäßrigen Lösungen durch freie Radikale als Starter basiert. Die antioxidative Effektivität ergibt sich durch die Inhibierungszeit im Vergleich mit einer Kontrolle. Diese schnelle und reproduzierbare Methode ermöglicht den Vergleich verschiedener Antioxidantien im Sudhaus. Korrelationen mit der Bieralterung und Vergleiche mit anderen Tests werden diskutiert werden.

Test global du pouvoir réducteur, en remplacement de la surveillance de l'activité lipoxygénasique du malt

Descripteurs
Activité enzymatique, analyse du malt, lipoxygénase, réducteur (chimie), stabilité organoleptique

RESUME
De récents travaux consacrés à l'affadissement de la bière ont fait apparaître le pouvoir réducteur comme facteur principal à surveiller tout au long du brassage et de la cuisson. L'activité lipoxygénasique du malt n'est pas le seul facteur conditionnant l'oxydabilité du moût. Une mesure mieux appropriée pour prévoir la stabilité de flaveur, basée sur l'oxydation de l'acide linoléique en solution aqueuse, induite par un initiateur, a été développée. L'efficacité antioxydante est donnée par le temps d'inhibition par rapport à un témoin. Cette méthode rapide et reproductible permet de comparer d'efficacité de divers antioxydants dans la chaudière. Les corrélations avec le vieillissement de la bière et des comparaisons avec d'autres essais sont étudiées.

INTRODUCTION

In order to avoid the release of a cardboard off-flavour through beer ageing, inhibiting lipid oxidation during mashing and brewing must be a constant concern for the brewers. Malt lipoxygenasic activity has often been correlated with the level of trans-2-nonenal in beer. However, as recently demonstrated (1), the nonenal potential created during mashing cannot survive after boiling. Therefore, only precursors issued from auto-oxidation will lead to beer staling.

Reducing power logically emerged as the key property to be controlled in the brewhouse. The reducing power of wort is often determined by colorimetric or electrochemical assays. Natural reducing substances in wort are mainly polyphenols extracted from both malt and hops and the products of Maillard reactions (reductones and melanoidins).

This paper aims at developping a new strategy for assessing the relative antioxidant properties of barley, malt, hop, wort and beer.

MATERIALS AND METHODS

Antioxidants - Aqueous solutions of antioxidants were prepared in 50 mM K-phosphate buffer (pH 7.4). Lipophilic antioxidants were first dissolved in pure methanol before a 50 times dilution by the same phosphate buffer containing 0.05 % v/v Tween 20.

Extraction of the antioxidants from malt and hop - Malt was finely ground in an EBC miller (Bühler-Miag) while hop pellets were crushed in a mortar. The ground samples (1g) were extracted four times under nitrogen with 7 mL methanol (HPLC grade) by shaking for 15 min and sonicating for 5 min. After centrifugation (3500 x g, 10 min), supernatant (25 mL) was collected. For the CO_2 hop extracts, an aliquot (1g) was dissolved under nitrogen in 25 mL methanol (HPLC grade) by sonicating for 10 min. Appropriate dilutions with pure methanol were applied taking into account a maximum methanol level of 0.33% (v/v) in the antioxidant assay.

Substrate preparation - An aqueous solution of linoleic acid (99%, Sigma) was prepared according to the method of Surrey (4). Substrate was stored at 4°C under argon until needed. Before use, the substrate was checked for auto-oxidation and solutions exhibiting more than 3 % auto-oxidation were discarded. The final concentration of linoleic acid in the substrate solution was 16 mM.

Antioxidant activity - The antioxidative activity is given by inhibition times of linoleic acid oxidation induced in an aqueous solution by a free radical initiator : 2,2'-azo-bis (2-amidinopropane) dihydrochloride (AAPH, Aldrich).

In a 3 mL quartz cell, 0.16 mM linoleic acid was oxidized by 2 mM AAPH in 50 mM K-phosphate buffer (pH 7.4) under air at 37°C. Oxidation was completed in the presence of either wort samples (dilution factor in test from 700 to 200), methanolic extracts of malt (final concentration in test of 133.3 ppm) or hop (final concentration in test from 1.7 to 16.7 ppm), or aqueous solutions of antioxidants (final concentration in test from 0 to 2.5 ppm). Lipid oxidation in the presence of the same level of methanol (max. 0.33%) was used for the assay without antioxidant (curve 0).

The rate of oxidation at 37°C was followed by the increase in absorption at 234 nm of the conjugated diene hydroperoxides. The Shimadzu UV-Visible 240 spectrophotometer equipped with an automatic sample positionner allows the analysis of six samples every minute.

RESULTS AND DISCUSSION

Oxidation of linoleic acid in aqueous phase and its inhibition by antioxidants

The oxidation of lipids initiated with AAPH (A-N=N-A) proceeds as follows (3):

Initiation:

$$A\text{-}N{=}N\text{-}A \rightarrow A\text{-}A + A^{\bullet} + N_2$$
$$A^{\bullet} + O_2 \rightarrow AO_2^{\bullet}$$
$$AO_2^{\bullet} + LH \rightarrow AOOH + L^{\bullet}$$

to mimic $LH + R^{\bullet} \rightarrow L^{\bullet} + RH$ (with $R^{\bullet} = OH^{\bullet}$ or $O_2^{-\bullet}$)

Propagation:

$$L^{\bullet} + O_2 \rightarrow LO_2^{\bullet}$$
$$LO_2^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

Termination:

$$2\ LO_2^{\bullet} \rightarrow \text{non-radical products}$$

with A : $-C(CH_3)_2-C(=NH)(NH_2)\cdot HCl$ and LH : linoleic acid.

In the absence of radical initiator, the rate of spontaneous oxidation at 37°C can be considered as negligible. The addition of AAPH induces an oxidation which starts at a constant rate of diene conjugated formation (*curve 0* in Fig.1a). When Trolox, a water-soluble analogue of vitamin E, is added, oxidation is delayed and an inhibition time can be determined (*curves 1-4* in Fig.1a). When the inhibition time is over, oxidation proceeds at the same rate as in the absence of inhibitor. As shown in Fig.1b, the inhibition time (Tinh) is proportional to the antioxidant concentration. Figures 2 and 3 give the slopes obtained in the same way for 9 well-known antioxidants.

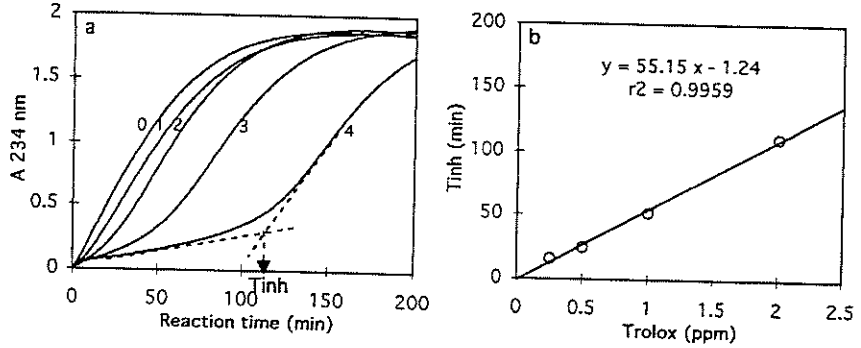

Fig.1 : a. Oxidation of 0.16 mM linoleic acid initiated with 2 mM AAPH in 50 mM K-phosphate buffer pH 7.4 under air, at 37°C, in presence of 0, 1, 2, 4 and 8 ppm Trolox (curves 0, 1, 2, 3 and 4, respectively).
b. Inhibition time as a function of Trolox concentration.

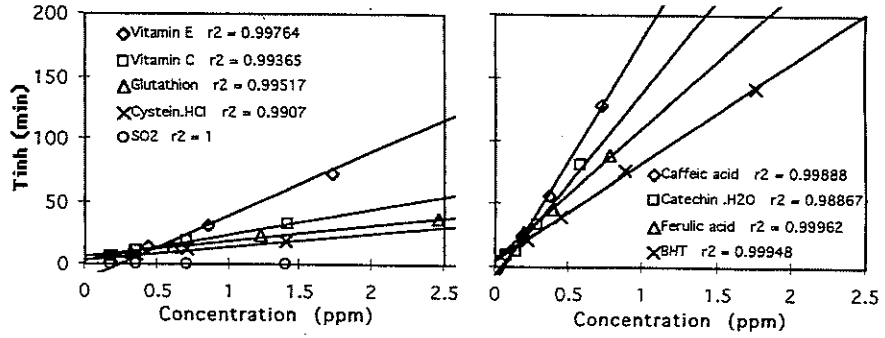

Fig.2 : Inhibition time as a function of antioxidant concentration.

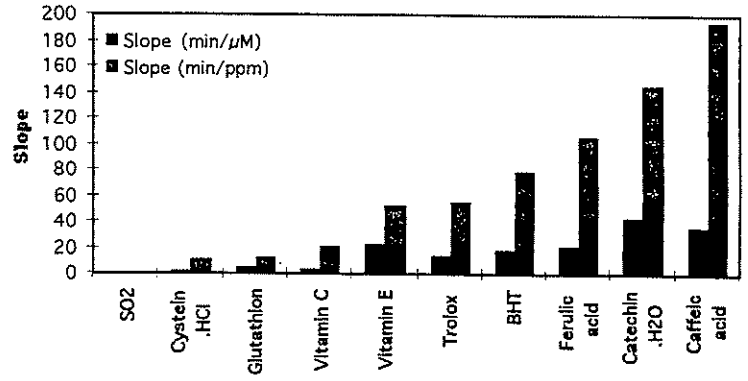

Fig.3 : Comparison of the efficiency of various antioxidants

Determination of the reducing power of worts

Although linoleic acid is the major fatty acid in wort, its occurrence will be negligible when the wort is diluted several hundred times. Fig.4 shows a linear relationship between the inhibition time and the reciprocal of wort dilution, as found above for pure antioxidants. Compared to the human low density lipoprotein (LDL) oxidation test proposed by Fantozzi et al. (2), our method gives quantitative results with a variation coefficient of 2.2 % (Table 1). Reducing power of the industrial wort (12°P) is described in Table 2 as equivalents of various reference antioxidants.

Table 1 : Reproducibility of the assay applied to an industrial wort (12°P) finally diluted 400 times.

Assay	Tinh (min)		Variation coefficient (%)
A	33.6		
B	34.8	**33.8**	2.2
C	32.8		
D	34.2		

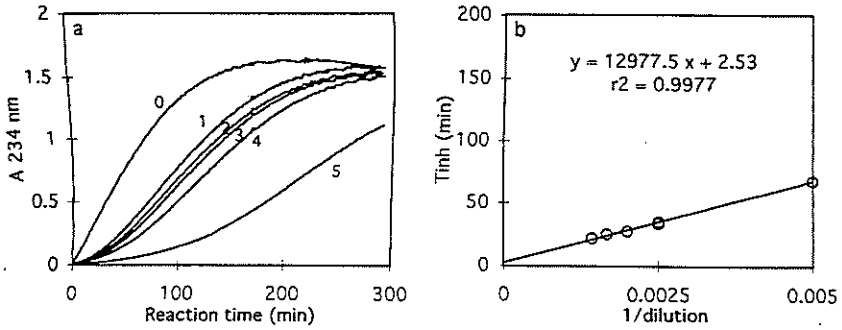

Fig.4 : a. Oxidation of 0.16 mM linoleic initiated with 2 mM AAPH in 50 mM K-phosphate buffer (pH 7.4) under air, at 37°C, in presence of increasing concentration of industrial wort. (curve 0 = without wort; curves 1,2,3,4 and 5 = 12°Plato wort diluted 700, 600, 500, 400 and 200 times, respectively).
b. Inhibition time as a function of 1/dilution in test.

Table 2 : Reducing power of an industrial wort (12°P) given in reference equivalents.

equivalents	ppm	mM
Cystein.HCl	1312	7.471
Glutathion	1080	3.515
Vitamin C	653	3.707
Vitamin E	262	0.609
Trolox	245	0.980
BHT	172	0.782
Ferulic acid	129	0.664
Catechin.H_2O	93	0.320
Caffeic acid	70	0.387

Determination of the reducing power of methanolic extracts of malts

Methanol was chosen as solvent medium for its capacity to dissolve both liposoluble and more hydrophilic compounds. Linoleic acid oxidation in presence of methanolic extracts of malt confirms the presence of peroxy radical scavengers in malt. Once more, the antioxidant activity is inversely proportional to the dilution ratio (data not shown). The procedure revealed to be more efficient with four successive 7 mL methanol extractions of 1g of malt (Tables 3 and 4).

Table 3 : Comparison between malt extraction procedures applied on 1g of pale malt. The final malt concentration in the assay is 133.3 ppm.

4 successive extractions of 7 mL					1 extraction of 28 mL			
	Tinh (min) *	**		Variation coefficient (%)		Tinh (min) *	**	
A	16.3 16.5	16.4			A'	14.0 13.8	13.9	
B	16.9 17.1	17.0	**16.4**	3.2	B'	14.7 14.5	14.6	**14.3**
C	14.6 16.8	15.7						

* assay or ** extraction duplicates

Table 4 : Efficiency of four successive 7 mL extractions applied on 1g of pale malt. The final malt concentration in the assay is 133.3 ppm.

Number of extractions	Tinh (min) *	
1	8.6 8.4	8.5
2	13.0 13.0	13.0
3	14.3 14.1	14.2
4	14.6 16.8	15.7

* assay duplicates

Determination of the reducing power of methanolic extracts of hops

The same method has further been applied on methanolic extracts of hops (Fig.5).

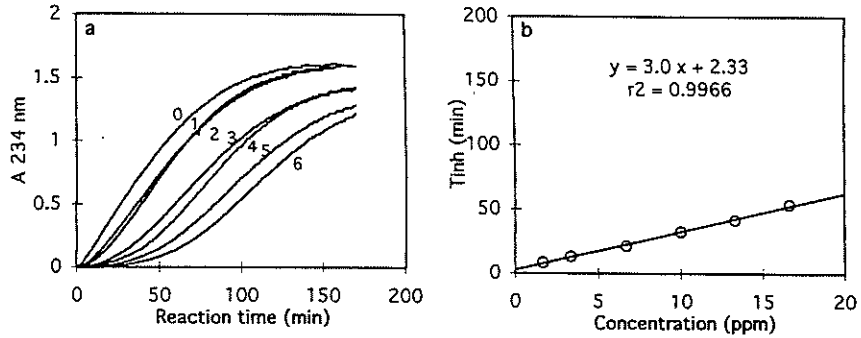

Fig.5 : a. Oxidation of 0.16 mM linoleic acid initiated with 2 mM AAPH in 50 mM K-phosphate buffer (pH 7.4) under air, at 37°C, in presence of 0, 1.7, 3.3, 6.7, 10, 13.3 and 16.7 ppm CO_2 hop extract (curves 0, 1, 2, 3, 4, 5 and 6, respectively).
b. Inhibition time as a function of final concentration in test.

Table 5 : Comparison between hop extraction procedures applied on 1g of hop pellets. The final hop concentration in the assay is 13.3 ppm.

4 successive extractions of 7 mL					1 extraction of 28 mL			
	Tinh (min) *	**		Variation coefficient (%)		Tinh (min) *	**	
A	50.1 50.2	50.1			A'	45.0 46.6	45.8	
B	49.9 52.0	51.0	**50.4**	1.7	B'	44.9 47.0	46.0	**45.9**
C	51.3	51.3			C'	45.8 46.9	46.4	
D	49.1	49.1			D'	43.7 47.5	45.6	

* assay or ** extraction duplicates

For pellets, the best results were also obtained when four successive 7 mL methanol extractions were applied (Table 5 and 6). As far as the inhibition of linoleic acid oxidation is concerned, hop appears in this assay 30 times more efficient than the malt tested above. Reducing power of a 1 ppm hop methanolic solution is expressed in Table 7 as equivalents of various references.

Table 6 : Efficiency of four successive 7 mL extractions applied on 1g of hop pellets. The final hop concentration in the assay is 13.3 ppm.

Number of extractions	Tinh (min) **	
1	28.6 32.7	30.7
2	39.3 45.3	42.3
3	45.1 51.5	48.3
4	49.1 51.3	50.2

** extraction duplicates

Table 7 : Reducing power of a 1 ppm hop methanolic solution given in reference equivalents.

equivalents	ppm	μM
Cystein.HCl	0.367	2.090
Glutathion	0.302	0.983
Vitamin C	0.183	1.039
Vitamin E	0.073	0.169
Trolox	0.069	0.276
BHT	0.048	0.218
Ferulic acid	0.036	0.185
Catechin.H_2O	0.026	0.090
Caffeic acid	0.019	0.105

CONCLUSIONS

A new reliable, rapid and low cost spectrophotometric method is proposed to measure the reducing power of raw materials, worts or beers. This method, based on the inhibition of lipid oxidation, gives informations about the efficiency of natural antioxidants to protect lipid oxidation *in situ*. Oxidation of exogenic linoleic acid by thermal free radical producer is followed by UV spectrophotometry in a highly diluted sample. The oxidation delay in presence of antioxidants is easily quantified owing to the high velocity of the initial reaction. Applied to malt, the method confirmed the presence of efficient antioxidants. However, such test revealed for the first time a much higher reducing power in hops. Our method emerges as very useful for exploring the oxidative changes during the brewing process and assessing the contribution of all raw materials.

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Production of an antioxidant extract from malt rootlets

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Descriptors
Antioxidant, extract, malt culms, phenolic compound, protein

SUMMARY
To prevent oxidation in beverages, cosmetics and foods, many studies are undertaken to find natural sources of antioxidants. So, two malt rootlets extracts have been produced: the first one contains 11,5% of the dry matter and is constituted of free compounds which are mainly proteins (45%), sugars (30%), and phenolic compounds (5%); the second one contains bound phenolic acids and represents 1,5% of the dry matter. The evaluation of their antioxidant activity in a lipidic medium at 60°C and their comparison with α-tocopherol and BHT point out the interest of the production of an antioxidant extract from malt rootlets.

Herstellung eines antioxidativen Extrakts aus Malzkeimlingen

Deskriptoren
Antioxidantium, Extrakt, Malzkeime, Phenolverbindung, Protein

ZUSAMMENFASSUNG
Zur Verhinderung von Oxidation in Getränken, Kosmetika und Nahrungsmittel wurden viele Studien durchgeführt, um natürliche Quellen für Antioxidantien zu finden. Es wurden zwei Extrakte aus Malzwurzelkeimen hergestellt: der erste enthält 11,5% der Trockensubstanz und besteht hauptsächlich aus Proteinen (45%), Zuckern (30%) und phenolischen Verbindungen (5%); der zweite enthält gebundene phenolische Säuren und besteht aus 1,5% der Trockensubstanz. Die Bewertung ihrer antioxidativen Aktivität in Ölen bei 60°C und der Vergleich mit α-Tocopherol und BHT zeigt das interessante Potential eines antioxidativen Extrakts aus Malzwurzelkeimen.