

### Communications en 2002, partie I

RAKOTOZAFY L., MENYE S., VIGNAUD C., BOUSSARD A. et NICOLAS J., (2002), Séparation de la cystéine et du glutathion et de leur forme oxydée par chromatographie liquide à haute performance couplée à une détection coulométrique. Optimisation par la méthodologie des surfaces de réponse. *Chimiométrie 2002*, 4-5 décembre, Paris, Poster. [Article complet](#) (pdf)

LOUARME L., BARTOLUCCI J-C., LUQUET M-P., POTUS J. et NICOLAS J., (2002), Effets du bromate, de l'iodate et du chlorate de potassium sur l'oxydation de la cystéine et du glutathion en solution aqueuse, sur la consistance des pâtes et sur le volume des pains. *53<sup>èmes</sup> Journées Techniques des Industries Céréalières*, 14-15 novembre, Paris, Poster.

LOUARME L., POTUS J et NICOLAS J., (2002), Oxydation de la cystéine et du glutathion par le bromate de potassium en solution aqueuse, en suspension de farine et dans la pâte boulangère. *53<sup>èmes</sup> Journées Techniques des Industries Céréalières*, 14-15 novembre, Paris, Poster.

VIGNAUD C, LOUARME L., POTUS J. and NICOLAS J., (2002), Disulfide formation catalysed by purified sulfhydryl oxidase (SOX) and wheat glutathione dehydroascorbate oxidoreductase (DHared) in thiol mixtures, comparison with the potassium bromate effect, *3<sup>rd</sup> European symposium on enzymes in grain processing*, 25-27 Septembre, Louvain, Poster. *Recent Advances in Enzymes in Grain Processing*, Ed. Courtin, C.M., Veraverbeke, W.S., Delcour J.A., (2003), 20, 127-132. [Abstract](#) : Nowadays, chemical bread improvers tend to be replaced by fungal enzymes. In this respect, we followed the thiol oxidation catalysed by sulfhydryl oxidase (SOX) purified from *A. niger* in presence of O<sub>2</sub>, and by glutathione dehydroascorbate oxidoreductase (DHared) purified from wheat flour in presence of DHA. These enzymatic thiol oxidations were then compared to the effect of potassium bromate. Different reaction mixtures containing glutathione (GSH, 0.25 - 0.5 - 1 or 2 mM), cysteine (CSH, 0.25 or 2 mM), SOX (or DHared) were studied at pH 5.6 (pH of the dough). HPLC equipped with an amperometric detector was used for the thiol assays whereas the produced disulfides were separated by size exclusion fractionation and quantified by an UV detector at 254 nm. With bromate and the 2 enzymes, 3 disulfides are produced GSSG, CSSC and the mix GSSC. In the early steps of the reaction, GSSG was the major disulfide obtained with SOX and DHared, but GSSC was produced in higher amounts with DHared. In addition, the obtained disulfide ratios depend on the relative concentrations of each thiol present in the mixture. However when the end point of the reaction was examined, the final disulfides ratios were the same whatever the system used. Therefore, for a given mixture of GSH and CSH, the three oxidizing systems led to the same final composition in disulfides. However, the equilibrium state was reached by different pathways since with the enzymatic systems, the GSSG disulfide was mainly produced in the beginning whereas the CSSC disulfide was preferentially produced with bromate.

RAKOTOZAFY L., FALGUIÈRES A., DOUSSOT J., GUY A. and NICOLAS J., (2002), Kinetic Studies of the Oxidation of Ferulic Acid Isomers and its Dehydrodimers by purified Peroxidases, *3<sup>rd</sup> European symposium on enzymes in grain processing*, 25-27 Septembre, Louvain, Poster. *Recent Advances in Enzymes in Grain Processing*, Ed. Courtin, C.M., Veraverbeke, W.S., Delcour J.A., (2003), 19, 121-126. [Abstract](#) : Ferulic Acid-*trans* (FA-*trans*) isomer accounts for 90 % of the total phenolic acids in wheat flour. FA units esterified to arabinoxylans can form dimers (diFA) by oxidative coupling catalyzed by enzymatic systems such as H<sub>2</sub>O<sub>2</sub> / peroxidase (POD) promoting by this way the oxidative gelation of pentosans in dough. In wheat flour, the most abundant diFA are 8-O-4', 8-5' benzofuran form, 8-5' and 5-5' dehydroferulic acid. Four diFA were synthesised according chemio-enzymatic methods, purified by gel filtration, and characterized according to their absorbance spectrum. Using an HPLC analysis coupled with a photodiode array detector, we showed that in model systems at pH 5.6 (pH of the dough), purified cationic peroxidases from wheat germ and horseradish

peroxidase produce predominantly the 8-5' benzofuran form diFA from FA-*trans* isomer and a different non identified diFA from the oxidation of FA-*cis* isomer. For both FA isomers, the 8-8'-g-lactone diFA was also formed. When the two isomers are mixed, the *trans* isomer is faster oxidized than the *cis*-isomer. The enzymes are also efficient to catalyze the oxidation of diFA 8-O-4', 8-5' benzo, 5-5' and 8-8'-g-lactone and the apparent constant kinetics towards two of those diFA have been determined by spectrophotometry. The enzymatic consumption of diFA or FA / diFA mixtures were also studied by HPLC but in this case the products could not be identified. Nevertheless, we can conclude that if H<sub>2</sub>O<sub>2</sub> is not limiting, the dimers are probably not the major coupling products of feruloyl polysaccharides in *vivo*.

LOUARME L., VIGNAUD C., RAKOTOZAFY L., POTUS J. and NICOLAS J. (2002), Kinetic study of thiol consumption by potassium bromate in aqueous solution, in water-flour suspension and in wheat dough, 3<sup>rd</sup> European symposium on enzymes in grain processing, 25-27 Septembre, Louvain,. Poster. *Recent Advances in Enzymes in Grain Processing*, Ed. Courtin, C.M., Veraverbeke, W.S., Delcour J.A. (2003), 47, 311-314. **Abstract** : Potassium bromate improves bread loaf, crumb, texture and increases process tolerance. It is generally admitted that its action consists in thiol oxidation during breadmaking. The aim of this study is to quantify bromate action on sulfhydryl groups, particularly on cysteine (CSH) and glutathione (GSH) in aqueous solution, in water-flour suspension and in wheat dough. Oxidation rates of thiols were determined using the Ellman's method and high performance liquid chromatography with electrochemical detection to follow the thiol consumption. In aqueous solution, CSH was oxidized approximately three times faster than GSH. A first order reaction rate was observed when the thiol and bromate concentrations were varied. The Q<sub>10</sub> of the reaction was close to 2 between 20 and 40 °C. When both thiols were present, their oxidation rates were hardly affected provided bromate was in excess. Conversely, in the presence of sulfhydryl oxidase (SOX, EC 1.8.3.3) and molecular oxygen and in the same conditions for the thiol concentration, CSH alone was very slowly oxidized whereas GSH was rapidly oxidized. However, when both thiols were present, a significant proportion of CSH was oxidized by SOX. Bromate reaction was also studied in water-flour suspension and in dough during mixing and during the rest period. Compared to the rates observed in aqueous solution, the oxidation rates of thiols decrease, but the ratio between CSH oxidation and GSH oxidation rates remains constant. Lastly, the thiol oxidation rates in dough were higher during mixing than during the rest period.

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