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NERON S., El AMRANI F., POTUS J. and NICOLAS J. (2004).**Separation and quantification by high performance liquid chromatography with light scattering detection of the main wheat flour phospholipids during dough mixing in the presence of phospholipase.** (2004)*Journal of Chromatography, Vol.1047,*p77-83.

Abstract
: Phospholipids are minor components of wheat flour involved in baking quality and exogenous phospholipids are used as emulsifiers giving better loaf volume and crumb grain. Few biochemical data are available on the phospholipids (PL) evolution during mixing, probably because of the time-consuming methods proposed for their extraction, separation and quantification. In the present study, the extraction, separation and quantification of the main wheat flour phospholipids were carried out. Total lipids (2 % dm of wheat flour) were extracted from flour or dough by a mixture of chloroform-methanol-water (1 : 1 : 1, v/v). The phospholipids were separated from the lipid extract on silica cartridge by solid phase extraction (SPE) procedure under a 1.5 to4 mm Hg vacuum, at a 0.8 $mL.min^{-1}$ flow rate. The recovery of the lipid extract was 100 %, whereas the SPE yield for the PL was 50 %. The resulting fraction was then submitted to an HPLC with evaporative light scattering detector (ELSD) on a Diol stationary phase allowing the separation and quantification of each class of phospholipids, in less than 16 min. The developed method allowed to quantify the phospholipid amounts from 8 wheat flours as well as their evolution during mixing in the presence of phospholipase.
Key words : phospholipids, HPLC-ELSD, SPE, wheat flour, phospholipase

GARCIA R., RAKOTOZAFY L.,NICOLAS J., (2004),**Analysis and modelling of the ferulic acid oxidation by a Glucose oxidase - Peroxidase association. Comparison with a Hexose oxidase - Peroxidase association.** (2004) *Journal of Agricultural and Food Chemistry,* 52, p-3946-3953. **Abstract**:A commercial glucose oxidase (GOX) from *Aspergillus niger* was partially characterized. The enzyme exhibited a two step transfer mechanism and the kinetic constants towards glucose and oxygen were determined. According to glucose levels in dough and to the environmental pH during mixing, GOX appears not to be in the best conditions to express its activity. Under conditions similar to dough making (glucose concentration and pH) GOX does not exhibit maximum activity. A hexose oxidase (HOX) from *Chondrus crispus* was partially characterized as well. The HOX activity is not far from the optimum in the kneading conditions (pH and glucose concentration). A peroxidase (POD) purified from wheat germ was used to oxidise ferulic acid in the presence of GOX or HOX. The Hydrogen peroxide produced during the glucose oxidation activates the wheat germ enzyme POD. Associations were made with different ratios of both enzymes and Ferulic acid oxidation in solutions containing different ratios of POD + GOX or HOX + POD were followed by UV spectrophotometry. For the same dosage, the HOX-POD system is the most efficient for peroxidase activation. Using absorbance data and kinetic constants of GOX and POD, a mathematical model describing the evolution release or consumption of the different reactants (hydrogen peroxide, oxygen and ferulic acid) in the medium has been established and a comparison between calculated values (using the model) and experimental data shows a good correlation. Experimental data correlated well with calculated values. These results could be of interest to understand glucose oxidase effect on the wheat dough rheological properties. The results obtained will be applied to investigate the effect of GOX and HOX activities on the rheological properties of dough. **Key words**: ferulic acid, peroxidase, bread making, glucose oxidase enzyme associations.

CAROLINE VIGNAUD, LALATIANA RAKOTOZAFY, ANNIE FALGUIÉRES, JACQUES POTUS, JACQUES NICOLAS (2004) **Separation and identification by gel filtration and HPLC-UV or HPLC-ECD of the disulphides produced from cysteine and glutathione oxidation**. *Journal of Chromatography A, Special issue : 27th International Symposium on High-Performance Liquid-Phase Separations and Related Techniques. Part II*-Edited by A.-M. Siouffi, Vol 1031/1-2 pp 125-133.**Abstract:**Methods for quantification of oxidized and reduced forms of glutathione (GSSG and GSH) and cysteine (CSSC and CSH) and the disulphide glutathione - cysteine (GSSC) resulting from the oxidation of the mixture

of CSH and GSH are performed by coulometric RP-HPLC and UV RP-HPLC after separation of these compounds by size exclusion FPLC. The fractionation of the disulphides (GSSG, GSSC and CSSC) was achieved by size exclusion using a Superdex peptide column coupled with an UV detection at 254 nm. The conditions of separation of these compounds by RP-HPLC were optimised using the response surface methodology (RSM). Optimal peak resolution and retention times were obtained on a C18 YMC ODS AQ column with 20 mM of ammonium phosphate at pH 2.5 and 2 % of acetonitrile in the elution phase. In these experimental conditions, CSH, CSSC, GSH and GSSG were eluted within 20 min. Coulometric detection enabled a sensitivity 100 times higher for the disulphides than the UV detection at 220 nm. These methods were applied to follow the consumption of thiols and the disulphide formation by three oxidising systems, sulphydryl oxidase (SOX), glutathione dehydroascorbate oxidoreductase (GSH-DHase) and potassium bromate. This study revealed that the relative proportions of the disulphides formed were similar for the three oxidising systems when the reactions are in their state of equilibrium. **Keywords** : glutathione, cysteine, size exclusion, RP-HPLC, coulometry, UV spectra, response surface methodology.

BILLAUD CATHERINE, MARASCHIN CHRISTELLE, NICOLAS JACQUES (2004). **Inhibition of polyphenoloxidase from apple by Maillard reaction products prepared from glucose or fructose with L-cysteine under various conditions of pH and temperature.** Lebensm.-Wiss. und Technol., **37**, 69-78. **Abstract*:***The effects of Maillard reaction products (MRPs), synthesized from equimolar glucose or fructose with L-cysteine (1 M) aqueous model mixtures, by modulating pH and temperature of heating, according to a 2 factor and five level experimental design, were investigated on polyphenoloxidase (PPO) activity from apple. Final pH and absorbance measurements at 350 nm were also selected as indicators of the Maillard reaction development and checked. In general, inhibitory potency of the mixtures increased with the increase in temperature (80-120 °C) and the decrease in pH (pH 2.0 to 12.0) of the reaction medium. A linear relationship between the inhibitory potency and heating time (0-48 h) or $Abs._{350\ nm}$ (0-70 A.U.) was demonstrated for glucose / cysteine system heated from 80 to 120 °C. Polarographic and spectrophotometric data were used to calculate kinetic constants and activation energy (Ea) values of inhibitory MRPs formation versus PPO activity and of those compounds absorbing at 350 nm. Ea values for these reactions were close, being 191 and 124 kJ ´ $mole^{-1}$ respectively. The experimental design allowed to conclude that linear effects of both factors as well as a quadratic effect of pH were significant, leading to optimum conditions for the production of glucose-derived MRPs inhibitors. In most cases, glucose produced MRPs with higher inhibitory potency compared to counterpart fructose-cysteine MRPs. **Keywords:**Maillard reaction; cysteine; apple PPO; inhibition.

SOPHIE BRUN-MÉRIMÉE, CATHERINE BILLAUD, LOàC LOUARME, JACQUES NICOLAS (2004). **Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition**. Food Chem., **84 (2)**, 235-241. **Abstract**:Maillard reaction products (MRPs) prepared from aqueous equimolar glucose or fructose with glutathione (GSH) model solutions (0.25 M), when heated at 90 °C for 15-39 h, were previously recognized as strong apple PPO inhibitors. This paper reports the inhibition mode of the purified *o*-diphenoloxidase activity from apple, using 4-methylcatechol as the substrate. Assuming a reversible inhibition, Lineweaver-Burk plotting showed that both MRPs were mixed-type inhibitors, glucose / GSH being the most efficient model system, with Ki values ranging between 11.6 and 1.1 µL MRPs, according to the heating treatment and the sugar tested. Enzyme inactivation was evidenced during pre-incubation (0-180 min) of PPO with various MRPs at 0 °C, only partly reversed by exhaustive dialysis or gel filtration. Activity initially lost was also partly restored when cupric ions ($CuSO_{4)}$ were added in the reaction medium, suggesting a chelating effect of copper ion at the active site of PPO, as already observed with cysteine-derived MRPs. MRPs derived from the tripeptide GSH revealed themselves more potent inhibitors of apple PPO than those prepared from cysteine. **Keywords** : Maillard reaction products, glutathione, apple PPO, inhibition kinetic, copper, chelating effect.

CATHERINE BILLAUD, SOPHIE BRUN-MÉRIMÉE, LOàC LOUARME, JACQUES NICOLAS (2004).**Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple. I. Enzymatic browning and enzyme activity inhibition**. Food Chem., **84 (2)**, 223-233. **Abstract**:The effect of unheated and heated glutathione (GSH) and Maillard reaction products (MRPs) derived from equimolar mixtures (0.25 M) glucose or fructose with GSH on purified apple polyphenoloxidase (PPO) activity was investigated by polarography and spectrophotometry, using 4-methylcatechol as the main phenolic substrate. When assayed alone, glutathione interacted with enzymatic-generated *o*-quinones giving rise to colourless conjugates, as demonstrated by high pressure liquid chromatography (HPLC). By polarography, increasing concentrations (0-300 mM) of GSH resulted in a high inhibitory effect on PPO activity, mostly due to a drop of pH of the reaction solutions to acidic values. Upon heating GSH at 90 °C, thermal degradation product formation was responsible for a partial PPO inhibition. GSH-derived MRPs highly inhibited PPO activity, inhibition efficiency increasing with heating time (2-39 h) and temperature (80-110 °C). Compared with MRPs prepared from hexose with cysteine, those from GSH exhibited a more potent inhibitory effect, due to the presence of an additional carboxylic function on the tripeptide. Therefore, these MRPs could be considered as potential natural inhibitors for use with minimally processed fruits. **Keywords :**Maillard reaction products, glutathione, PPO, enzymatic browning, enzyme inhibition.

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