XVII[th] World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

MICROWAVE-ASSISTED METHODOLOGY FOR QUANTITATIVE ANALYSIS OF LIGNANS IN FOODS

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CSBE100781 – Presented at Section VI: Postharvest Technology and Process Engineering Conference

ABSTRACT Lignans with dibenzylbutanediol structure, such as the flaxseed lignan secoisolariciresinol, have been shown to benefit human health. They have antioxidant properties and the potential to lower the risk of developing chronic diseases such as diabetes and breast cancer. There has been increasing interest in the identification and quantification of lignans in foods. This has materialized in publications reporting lignan contents in various foods, and a free electronic database that compiles lignans data from peer-reviewed articles. However, the precise quantification of lignans remains challenging. The main limiting steps for a quantitative recovery of lignans are the choice of extraction methodologies and the extract purification procedures. Extraction with aqueous alcohols can take anywhere from 1 to 48 h without insuring a complete recovery of lignans from the food matrix. Due to the fact that plant lignans naturally occur conjugated to a variety of sugars, fatty acids, or are incorporated into complex polymeric structures, a hydrolysis step is required. Alkaline hydrolysis is robust, efficient and the method of choice for flaxseed lignan analysis. However, it is not sufficient for other grains where the lignans are conjugated to unknown sugars. Most food grains require acidic or enzymatic hydrolyses in order to free the lignans from their conjugated states. Acid hydrolysis leads to the formation of lignan artefacts thus complicating the subsequent chromatographic analysis. Enzymatic hydrolysis is less damaging than the acidic hydrolysis but conversion of lignan glucosides to aglycones might not be complete. The purification of lignan extracts is necessary before either enzymatic hydrolysis or chromatographic analysis, or in both cases. The purification by liquid-liquid extraction is hampered by the reduced solubility of lignan glucosides in non-aqueous organic solvents. The purification by reversed-phase solid phase extraction is preferred as it can be applied to both lignan aglycones and glucosides. Due to the complexity of lignans analysis in foods, every step of the extraction and purification methodologies has to be optimised in order to maximise lignans recovery. This project proposes a step by step optimization involving microwave-assisted extraction followed by solid phase extraction purification and enzymatic hydrolysis. The scope of the project is to identify the extraction parameters that are optimal for lignan extraction from a variety of food grains. Initially, an optimized microwave-assisted methodology has been developed, by applying response surface experimental procedures. The microwave-assisted extraction has increased the

lignans recovery from flaxseed by 6% as compared to a conventional direct hydrolysis, and by more than 20% as opposed to sequential methodologies involving alcoholic extraction followed by alkaline hydrolysis, or alkaline hydrolysis followed by acidic hydrolysis. Next, response surface experiments were conducted in order to assess the effects of purification of extracts, the molar concentration of buffer, the concentration of enzyme and the time of incubation on the yield of conversion of lignan glucosides to aglycones. The optimized extraction methodology has been used for quantifying the amounts of secoisolariciresinol and its glucoside in various flaxseed cultivars from Quebec and in food grains available on the market such as black and white sesame, chia (salba), quinoa seeds and beans.

Keywords: Microwave-assisted extraction, lignan quantification, secoisolariciresinol, flaxseed.