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# Effect of hydrothermal processing on changes of insoluble-bound phenolics of lentils

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ABSTRACT

The changes in the insoluble-bound phenolics (IBPs) of lentil cultivars upon hydrothermal (boiling) processing were monitored using HPLC-ESI-MSn analysis and by following different antioxidant capacity measurements. The hydrothermal energy disintegrated or loosened the cell wall matrix, hence the content of insoluble-bound phenolics decreased as shown by HPLC analysis and measurement of phenolics and antioxidant capacity during the boiling process, indicating their possible release from cell wall matrix. However, the released bound phenolics did not remain as soluble/free phenolics (SPs) as their increase was less than the decrease in bound phenolics, indicating loss of phenolic compounds. This loss is speculated to be due to the formation of irreversible covalent bonds to other molecules such as protein, starch, and cellulose, which are not affected by alkali hydrolysis procedure used in this work. Thus, structural and positional changes of bound phenolics and possible alteration of chemical bonding during boiling may be contemplated.

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## 1. Introduction

Lentils serve as a valuable dietary source of protein, carbohydrate, minerals, vitamins, and dietary fibre (Rochfort & Panozzo, 2007), thus provide essential nutrients to many populations around the world, especially the vegetarians. Recently, several studies on the antioxidant capacity of lentils have demonstrated their excellent radical scavenging potential in *in vitro* systems such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS), and hydroxyl radicals as well as reducing power (Alshikh, de Camargo, & Shahidi, 2015; Xu & Chang, 2008; Yeo & Shahidi, 2015; Zhang et al., 2015).

Phenolic compounds, based on their solubility in the extraction medium, can be divided into two groups, namely soluble phenolics that include both the free and esterified phenolics and insoluble-bounds phenolics. Most of the soluble phenolics are localized in vacuole of plant cells and are trapped by weak interaction with other compounds (Li et al., 2012), whereas insoluble-bound phenolics are localized in the cell wall matrices through covalent bonds. The mechanism of the formation of insoluble-bound phenolics has not yet been well discussed in the food science field (Yeo & Shahidi, 2016). However, other research areas such as biology have investigated their synthesis, transfer, and formation in a variety of plant-based foods at a cellular level. In the formation of insoluble-bound phenolics, the synthesized phenolic compounds in the intracellular organs, mainly endoplasmic reticulum, are released and transported to the cell wall matrices through the vesicles transfer system, facilitating migration of phenolic compounds into the cell wall (Meyer, Pajonk, Micali, O'Connell, & Schulze-Lefert, 2009). The transported phenolic compounds are bound to the insoluble macromolecules such as protein, cellulose, and pectin through covalent bonding, including ether, ester, and carbon-carbon bonds in the cell wall matrices in which they play a significant role in building cell wall matrices and protecting from outer predators such as pathogens and insects (Dai et al., 1996; Nicholson & Hammerschmidt, 1992).

The insoluble-bound phenolics are not absorbed in the small intestine, since they are covalently bound to the insoluble macromolecules, leading to their transfer to the large intestine (colon) where they are fermented by a number of microorganisms, followed by liberation of insoluble-bound phenolics due to the cell wall disintegrating enzymes such as cellulase, amylase, and pectinase released by the microorganisms. The released phenolics act as health promoting compounds by influencing the colon environment such as pH, leading to inhibition of the growth of harmful bacteria. Meanwhile, soluble phenolics can be absorbed in the digestive track and their effectiveness in the biological system is depend on their bioavailability and bioaccessibility since the structure of phenolics are generally altered during absorption and transportation in the blood stream. Thus, investigation on the bioavailability and bioaccessibility of phenolics would provide a valuable information about the effectiveness of the phenolics in the biological system (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014).

Boiling is the most common cooking method for improving the acceptability, texture, and nutritional value of legumes as well as eliminating anti-nutritional factors (Shahidi & Naczk, 2004). A number of studies have reported the changes in the content of phenolic compounds and antioxidant capacity of plant-based foods by heat treatments such as boiling. For example, boiling was found to reduce the content of phenolic compounds such as caffeic acid, gallic acid, apigenin-7-O-glucoside, ferulic acid, syringic acid, isovitexin and phloridzin in fennel (Rawson, Hossain, Patras, Tuohy, & Brunton, 2013). Boiling process also decreased the antioxidant capacity in total phenolic content and DPPH radical scavenging activity of green pea, yellow pea, chickpea and lentil (Xu & Chang, 2008) and attenuated the antioxidant capacity of faba beans (Siah et al., 2014). However, most of these studies only reported changes in the soluble phenolics, with a few considering the insoluble-bound phenolics. Therefore, studies on the changes of antioxidant capacity and content of insoluble-bound phenolics of legumes are needed in order to fill the existing gap in the available knowledge in the field.

The aims of this study were to (1) monitor changes of antioxidant capacity of insoluble-bound phenolics in four lentil cultivars during boiling and (2) determine the profiles of insoluble-bound phenolics and their contents using HPLC-ESI-MS/MS.

## 2. Materials and methods

### 2.1. Materials

Lentils cultivars, namely CDC green land, CDC invincible, 3493-6, and maxim were provided by Professor Albert (Bert) Vandenberg of the experimental farm of the University of Saskatchewan, Saskatoon, Canada. Hexane, methanol, acetone, ethanol, sodium carbonate, sodium chloride, and sodium hydroxide were purchased from Fisher Scientific Co. (Nepean, ON, Canada). Other chemicals such as, Folin and Ciocalteau's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallocatechin, gallic acid, and catechin were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). The compound 2,2 azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein, trichloroacetic acid (TCA), potassium ferricyanide [$K3Fe(CN)_6$], sodium nitrite ($NaNO_2$), and aluminum chloride ($AlCl_3$) were purchased from Sigma-Aldrich Canada Ltd.

### 2.2. Boiling (hydrothermal) process

Ten grams of each type of lentils were well washed with distilled water and placed in 125 mL flask containing 50 mL boiling distilled water and then continuously boiled for 25 min using a hot plate. After 25 min, most of the distilled water was evaporated (in this procedure most of phenolics might be adsorbed to the lentils) and subsequently cooled at ambient temperature. The boiled samples were then freeze-dried and the samples were collected into sample bags, followed by storage at −18 °C for the further use.

### 2.3. Extraction of soluble phenolics (SPs) and insoluble-bound phenolics (IBPs)

The extraction of SPs and IBPs was carried out as described by Yeo and Shahidi (2015). Briefly, the freeze-dried boiled lentils were finely ground and subsequently defatted by using n-hexane. For the extraction of SPs, 1 g of defatted sample was mixed thoroughly with 10 mL of a solution of methanol/acetone/water (1:1:1, v/v/v) over 20 min at room temperature. This procedure was repeated two more times and the combined extracts were used for determination of **SPs. The** residue after removal of **SPs** was used for the extraction of IBPs. For the extraction of IBPs, 1 g of the residue (after extraction of SPs) was mixed with 15 mL of 2 mol/L NaOH to fill the vial completely and allowed to stand for 4 h while stirring. Hydrolyzed samples were acidified with 4.63 mL of 6 mol/L of HCl (in order to make pH of <2 for the better extraction of hydrolyzed phenolics) and then the hydrolyzed phenolics were extracted with ethyl acetate five times. Subsequently, the solvent was removed using a rotary evaporator, followed by dissolving the resultant solids in methanol/acetone/water (1:1:1, v/v/v).

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