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Impact of rapeseed press-cake on Maillard reaction in a cookie model system

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ABSTRACT

Rapeseed press-cake (RPC) is a byproduct of rapeseed oil production, rich in proteins and fiber. The aim of this study was to investigate the impact of cold pressed RPC, RPC fiber isolate and RPC alkaline extract on the formation of acrylamide and 5-hydroxymethylfufural (HMF) in cookies. Both compounds were influenced by the ingredients: the addition of RPC led to a significant dose-dependent increase of HMF in the cookies and to an increase of acrylamide up to 66.9%. On the contrary, acrylamide concentration was reduced down to 39.6% in presence of the alkaline extract and down to 4.4% in the presence of the fiber extract. The Michael addition of free amino acids to acrylamide was further investigated by high-resolution mass spectrometry (HRMS) revealing that cysteine was the preferred nucleophile for acrylamide elimination.

1. Introduction

The worldwide production of rapeseed increased by a factor of 2.4 over the last 20 years thus making rapeseed the most cultivated oilseed crop in Europe with a production of 19.5 M tons in 2012. The increase in rapeseed production is due to two major aspects: the expansion of biodiesel production from rapeseed and the improvement of the nutritional composition (e.g. decrease of erucic acid levels and glucosinolate levels) of rapeseed seeds (EFSA, 2013). Rapeseed press-cake (RPC) is a byproduct of rapeseed oil production and is mainly used in animal feed. However, RPC contains notable amounts of phenolic acids and tannins, as reviewed by Naczk and coworkers (1998); cold-pressed RPC is rich in crude fiber (around 11.2%) and protein (approximately 28.0%) hence having high potential to be used in human diet (Leming & Lember, 2005). Indeed, a novel rapeseed protein isolate extracted from canola varieties (*Brassicaceae napus* L. and *Brassicaceae rapa* L.) was adjudged to be safe and suitable for human diet by the EFSA (EFSA, 2013). Palermo and co-workers (2012) showed that the addition of a similar product based on soy proteins (okara) to a cookie model system was positively correlated to higher concentrations of acrylamide (+60%), *N*ε-(carboxymethyl)-L-lysine (+400%), and HMF (+100%). These compounds are commonly used as markers of the Maillard reaction (MR) that along with lipid oxidation, sugar caramelization and ascorbic acid oxidation, occupies a prominent place in nonenzymic browning. Beside the formation and degradation of the Amadori compounds, during the intermediate and advanced stage of the reaction, cyclization, dehydration, retroaldolization, rearrangement, isomerization, and further condensation take place. In the final/advanced stage, melanoidins and brown pigments are formed (Hellwig & Henle, 2014).

The Maillard cascade leads to hundreds of molecules, some of these compounds are useful since they are responsible for color and flavor formation or they contribute to texture. Some other Maillard reaction end products (MRPs) can contribute to the off-taste of foods or have potential negative effects on human health; an example is the formation of acrylamide and HMF which are two of the most-studied MRPs (Capuano & Fogliano, 2011).

Acrylamide is formed upon the reaction of asparagine with reducing sugars or from cleavage products (i.e., 2-butanedione, 2-oxopropanal) at temperature higher than 100 °C in low moisture conditions or after prolonged thermal treatment (Mottram, Wedzicha, & Dodson, 2002). Cysteine and methionine in presence of reducing carbonyls and acrolein in presence of ammonia are other precursors of acrylamide, even if the yield is lower than the asparagine/reducing sugars system (Stadler et al., 2002). Focusing on the asparagine/glucose system, there are several routes that lead to acrylamide formation. The first mechanism involves 3-aminopropionamide as reaction intermediate upon the formation of the Schiff base and the subsequent decarboxylation and

Abbreviations: RPC, rapeseed press cake; MR, Maillard reaction; HMF, 5-hydroxymethylfurfural; HRMS, high resolution mass spectrometry; LC, liquid chromatography; WHC, water holding capacity; TPC, total phenolic content; BSA, bovine serum albumin

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hydrolysis via the Strecker degradation (Granvogl, Jezussek, Schieberle, & Koehler, 2004). The second mechanism includes the direct decomposition of the Schiff base via intramolecular cyclization forming the azomethine ylide that directly decomposes on cleavage of the C-N bond to give acrylamide and 1-amino-2-hexulose (Yaylayan, Wnorowski, & Perez Locas, 2003). Due to the presence of an acryloyl group, acrylamide is highly reactive and it can polymerize or form Michael adducts with free amino group, thiols or other nucleophiles (Adams, Hamdani, Lancker, Méjri, & De Kimpe, 2010; Koutsidis et al., 2009). Acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC) and a Category 2 carcinogen and Category 2 mutagen by the European Union; moreover the EFSA mentions acrylamide formation in foods as one of their major concerns (Friedman, 2015).

HMF is used as an indicator for the heating of carbohydrate containing foods (Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2000). Several mechanisms concur to its formation since it can arise from both caramelization and MR (Hollnagel & Kroh, 1998). HMF is formed as an intermediate from 1,2-enolization and dehydration of glucose or fructose under acidic conditions. The further dehydration and cyclization of 3-deoxyosone is the key step for the formation of HMF. In acidic conditions, HMF is mainly formed at high temperature under dry and pyrolytic conditions; it can arise from fructose and sucrose via the formation of highly reactive fructofuranosyl cations (Perez Locas & Yaylayan, 2008). Beside the increase of temperature, there are other factors that influence the formation of HMF: the pH, the water activity (Oliviero, Capuano, Cämmerer, & Fogliano, 2009) and the dehydration promoted by bivalent cations (Gökmen & Şenyuva, 2007). HMF shows mutagenic, hepatotoxic and carcinogenic effects *in vitro* even though a toxic effect in humans has not been confirmed, as yet. A daily exposure of 2 to 30 mg of HMF per person through a regular diet has been estimated, suggesting its reduction in foods as a relevant issue (Capuano & Fogliano, 2011).

In this paper, the effects of three different ingredients obtained from RPC were investigated in order to improve the quality of cookies by monitoring the formation of acrylamide and HMF through the use of a food byproduct. In this respect, the acrylamide elimination via Michael addition was evaluated by liquid chromatography high-resolution mass spectrometry (LC-HRMS) to tentatively identify the mechanisms beside acrylamide elimination.

2. Materials and methods

2.1. Chemicals

Cold pressed native rapeseed cake pellets from *Brassica napus* L. var. *catana* were provided by Summer Harvest® oil manufacturer Perthshire (UK), and were stored in an airtight closed plastic bag at room temperature. Wheat flour and sugar in customary quality were obtained from local stores. Palm oil was purchased from the Kerfoot Groop, (Yorkshire, UK). All the other baking ingredients were of analytical grade and purchased from Sigma-Aldrich (Saint Louis, MO). All chemicals used in this study were purchased from Sigma-Aldrich (Saint Louis, MO) and were of analytical grade, unless mentioned otherwise.

2.2. Ingredients extraction

2.2.1. Alkaline extraction

Alkaline soluble protein extraction was performed following a procedure previously described by Ghodsvali, Khodaparast, Vosoughi, and Diosady (2005) with some modification. Briefly, freshly ground native RPC powder was weighed into a beaker and distilled water was added at a w/v ratio 1:20. The pH was adjusted to 11 with NaOH (0.1 M) and the suspension was stirred for 30 min at room temperature. After centrifugation (25 min, 0 °C, 2058*g*) the supernatant was collected and adjusted to pH 6.5 with HCl (0.1 M). The precipitated pellet was separated by centrifugation (25 min, 0 °C, 2058*g*) and the supernatant was collected. A second precipitation was conducted by adjusting the pH to 4.5. The pellets of both precipitation steps were merged and washed two times at a w/v ratio 1:3 with distilled water and freeze-dried.

2.2.2. Fiber extraction

The dietary fiber was obtained from the pellet which remained after the first extraction step of the alkaline soluble protein extraction. The pellets were washed 6 times with distilled water at ratio of 1:2.5 w/v and freeze-dried.

2.2.3. Water holding capacity (WHC) determination

In order to determine the WHC, 1 g of the fiber was weighed into a centrifuge tube and mixed vigorously with 50 mL distilled water for 1 min. The mixture was centrifuged for 40 min (2058*g* at 20 °C). The supernatant was discarded and the centrifuge tube was kept inverted for 10 min. The supernatant was weighed and the results were expressed as grams of water per gram of fiber (Chen, Rubenthaler, Leung, & Baranowski, 1988).

2.3. Preparation of the cookies

The cookies were prepared according to a recipe described in an American Association of Cereal Chemists (AACC) method 1054. The effect of the components from rapeseed press-cake was evaluated by replacing 6.5% and 12.9% of the flour content with the alkaline extract, 1.3%, 2.6%, and 5.2% of the flour content with the fiber extract, and 6.5% and 12.9% of the flour content with the native RPC, respectively (Table 1). Each dough was rolled between two bars with a height of 3 mm and were shaped in a disk of 30 mm diameter. To ensure repeatability of the experiment, the same amount of dough (17 g) was placed on the middle shelf of a laboratory oven (Memmert UM200, Schwabach, Germany) and in the center of the shelf and baked at 180 °C for 18 min.

2.4. Spectrophotometric assays

A methanol extract (60% methanol) of the ground RPC was prepared at a w/v ratio of 1/10 (v/v) to determine the antioxidant capacity and the total phenolic content (TPC) of the rapeseed press-cake. In order to determine the content of soluble protein using Bradford assay the extracts were prepared the same way replacing the methanol with distilled water. The suspensions were subsequently centrifuged (2058*g*, 10 min, 4 °C), and supernatants were diluted if necessary.

Table 1
Recipes used for the different cookies.

	Control cookies	Alkaline extract cookies	Fiber extract cookies	RPC cookies
Wheat flour (g)	80.0	70.0 and 60.0, respectively	78.0; 76.0, and 72.0, respectively	70.0 and 60.0, respectively
Alkaline extract (g)	/	10.0 and 20.0, respectively	/	/
Fiber extract (g)	/	/	2.0; 4.0, and 8.0, respectively	/
Native press-cake (g)	/	/	/	10.0 and 20.0, respectively
Palm oil (g)	20.0	20.0	20.0	20.0
Sucrose (g)	35.0	35.0	35.0	35.0
$NaHCO_3$ (g)	0.8	0.8	0.8	0.8
NaCl (g)	1.0	1.0	1.0	1.0
NH_4HCO_3 (g)	0.4	0.4	0.4	0.4
Water (ml)	17.6	17.6	17.6	17.6