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Using LC-MS to examine the fermented food products vinegar and soy sauce for the presence of gluten

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ABSTRACT

A strict, lifelong gluten-free (GF) diet is currently the only treatment for coeliac disease (CD). Vinegar and soy sauce are fermented condiments that often include wheat and/or barley. During fermentation cereal proteins are partially degraded by enzymes to yield peptide fragments and amino acids. Whether these fermented products contain intact or degraded gluten proteins and if they are safe for people with CD remains in question. LC-MS offers the benefit of being able to detect hydrolysed gluten that might be present in commercial vinegar and soy sauce products. LC-MS revealed the presence of gluten in malt vinegar, wherein the identified peptides derived from B-, D- and γ-hordein from barley, as well as γ-gliadin, and HMW- and LMW-glutenins from wheat that are known to contain immunopathogenic epitopes. No gluten was detected in the soy sauces examined despite wheat being a labelled ingredient indicating extensive hydrolysis of gluten during soy sauce production.

1. Introduction

Coeliac disease (CD) is a *T*-cell mediated auto-immune disorder triggered by ingestion of cereal gluten found in wheat (gliadins and glutenins), barley (hordeins) and rye (secalins). Clinical symptoms of CD are diverse and include flatulence, bloating, fatigue, indigestion, diarrhoea, abdominal distension/pain, weight loss, low bone mineral density, anaemia, irritability, anxiety, depression and neurological disorders (Green, et al., 2003). CD affects approximately 70 million people or ~1% of the world's population (Fasano et al., 2003) and ~10% are affected by non-coeliac gluten sensitivity (NCGS) (Aziz et al., 2014; Golley, Corsini, Topping, Morell, & Mohr, 2015). For both conditions strict avoidance of gluten is the recommended treatment.

Vinegar and soy sauce are food ingredients used to impart flavour. There are various types of vinegar, including distilled vinegar, fruit-based vinegar (cider, apple, grape and wine), rice vinegar and malt vinegar. Only malt vinegars produced by fermentation of cereals containing gluten, commonly malted barley and wheat, are of concern to people with CD. Soy sauce is traditionally made from a fermented paste of soybeans, roasted grains (commonly wheat), brine and *Aspergillus* moulds (commonly *A. oryzae* or *A. sojae*). During fermentation, the gluten proteins are partially hydrolysed into peptides. If these protein fragments contain immunopathogenic epitopes (Sollid, Qiao, Anderson, Gianfrani, & Koning, 2012), the consumption of these food ingredients may cause adverse reactions in those with CD.

Gluten-free (GF) is defined by the Food and Drug Administration (FDA) and Codex Alimentarius as < 20 mg/kg (CODEX, 2008; Food and Drug Administration, 2013). Enzyme-linked immunosorbent assays (ELISA) are the currently accepted method for gluten detection in food. However, questions remain around the ability of ELISA to detect the hydrolysed forms of gluten (Panda et al., 2015; Slot, Bremer, van der Fels-Klerx, & Hamer, 2015; Thompson & Mendez, 2008).

Gluten detection using liquid chromatography mass spectrometry (LC-MS) has been applied to the analysis of beer, a fermented beverage (Allred, Sealey Voyksner, & Voyksner, 2014; Colgrave, Goswami, Blundell, Howitt, & Tanner, 2014; Picariello et al., 2015; Tanner, Colgrave, Blundell, Goswami, & Howitt, 2013). Cumulatively, these studies have demonstrated that LC-MS is capable of detecting hydrolysed or modified gluten that may evade detection by ELISA (Haraszi, Chassaigne, Maquet, & Ulberth, 2011). LC-MS has not yet been applied to other fermented products such as vinegar and soy sauce, but these products have been examined by ELISA. In a recent study, the gluten content of soy sauce was monitored over the fermentation process using a suite of immunochemical methods including both competitive and sandwich ELISA kits and no gluten could be detected after the second stage of fermentation (Cao et al., 2017). The same group developed a

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Table 1
Identification of gluten proteins and peptides in malt vinegar after different sample preparation strategies. Spectral datasets were searched against the Poaceae subset of the Uniprot database. The protein score, sequence coverage, protein name (and accession), peptide sequence and mass error (ppm) are given.

N	Protein Score	% Coverage (95%)	Protein Name	Species	Sequence	ΔM (ppm)
13	7.25	6.38	HMW-GS Ax2 (Q41553)	*T. aestivum*	AQGQQGQQPER[b]	−2.49
					QQDQQSGQGQQPGQR	−0.37
					QQPGQGQQLR	0.86
					YYPTSPQQPGQEQQPR	1.54
14	6.92	5.68	D-Hordein (I6TRS8)	*H. vulgare*	CCQQLR[b]	−2.90
					DVSPECR[b]	−3.68
					DVSPECRPVA[b]	−0.97
					DVSPECRPVALSQVVR	−0.13
					ELQESSLEACR	1.29
					YEQQTEVPSK[b]	−0.61
16	6.59	15.71	Farinin protein[a] (W8QN23)	*B. distachyon*	GEQHSSCQTVQHQCCR	3.11
					HTLPSMCK[b]	−2.16
					QLAQIPEQFR	−1.59
					QLVQIPEQAR	−1.65
19	5.5	14.58	γ3-hordein (I6TEV2)	*H. vulgare*	CPAIQTIVH[b]	−0.94
					EQHQLNLCK[b]	−1.04
					ISQQNSCQLK[b]	−0.30
					QQCCQQLANINEQSR	0.26
23	4.09	7.92	HMW-GS (W6AX70)	*T. aestivum*	QPGQGHPGQR[b]	−2.22
					QQPGQGQQTR	−1.84
					QVVDQQLAGR	−0.88
38	2.25	3.80	HMW-GS 1Bx14 (Q45R38)	*T. aestivum*	DVSPGCRPITVSPGTR	1.29
					SQQSEQGQQPGQGK[b]	0.08
41	2.03	6.43	γ-gliadin (B6DQD5)	*T. aestivum*	NILLQQCKPAS[b]	−1.25
					QQCCQQLAR	1.23
44	2.00	6.19	HMW-GS 1Dy (P10387)	*T. aestivum*	QQPGQGQHPEQGK	0.70
50	2.00	2.86	LMW-GS (P16315)	*T. turgidum*	QLPQIPEQSR	−0.10
56	2.00	3.79	B-hordein (I6TMW0)	*H. vulgare*	VFLQQQCSPVR	−1.26
6[c]	1.36	5.08	LMW-GS (R4JMD1)	*T. aestivum*	SHHQQQQPIQQQPQPF	−1.37

[a] Farinin protein from *Brachypodium distachyon* (purple false brome) sharing homology with wheat avenin-like proteins.
[b] Peptides that are not fully tryptic, i.e. these are non-specific hydrolysis products resulting from brewing.
[c] Peptide identified after chymotrypsin digestion.

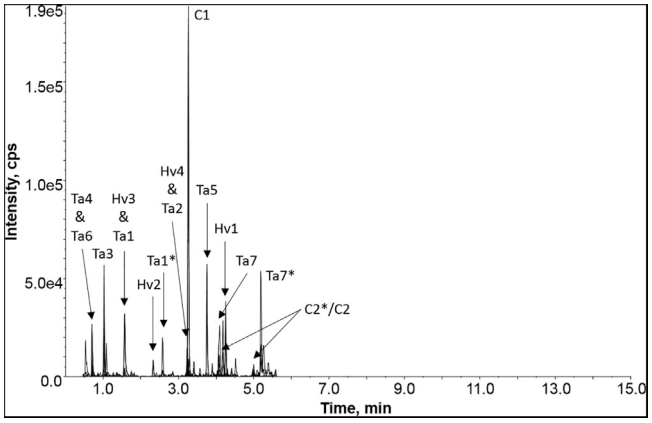

Fig. 1. LC-MRM-MS analysis of malt vinegar confirms barley and wheat as ingredients and the presence of gluten. The LC-MS chromatogram showing the extracted ion chromatograms (XIC) for gluten peptides (Table 2) from barley (Hv1-Hv4), wheat (Ta1-Ta7) and two peptides that are common to barley and wheat. An asterisk* refers to a modified peptide.

multiplex competitive ELISA using nine commercial antibodies and used this assay to assess a range of fermented-hydrolysed foods (Panda, Boyer, & Garber, 2017). The results indicated the high level of variation between the different commercial antibodies. Additionally, they demonstrated that analysis of food products containing high levels of salt, sugar or other additives, such as soy sauce and vinegar, may be problematic due to non-specific inhibition that prevented the antibodies binding to the coated gluten leading to apparent false positives. The multiplexed assay was however capable of distinguishing foods on the basis of their type and degree of hydrolysis. The objective of this study was to examine vinegar and soy sauce for the presence of gluten by using an alternative analytical approach, LC-MS.

2. Materials and methods

2.1. Sample collection

Two types of vinegar, a distilled and a malt vinegar, with barley and wheat as labelled ingredients, and 10 commercial soy sauces, three GF and seven containing wheat, were obtained from a local supermarket in Australia. Three batches of the malt vinegar were purchased as judged by the expiry date. The 'gluten-free' label in Australia requires that the product contains no detectable gluten nor wheat, barley, rye or their hybrids as ingredients which differs from other jurisdictions wherein the threshold is 20 mg/kg.

2.2. Reference materials

Gliadin reference material (PWG-gliadin) was supplied by the Prolamin Working Group (PWG) (van Eckert et al., 2006). The PWG-gliadin was prepared as per manufacturer's instructions to yield stock solutions of 10,000, 1000 and 160 mg/L in 60% aqueous ethanol. A series of incurred soy sauces (n = 4 replicates) were prepared by