

Appendix D.

Literature search of confocal laser scanning microscopy (CLSM) papers that have examined gluten's structure within food systems

In Chapter 4, method development for the CLSM method was undertaken due to the lack of consensus and explanation throughout the literature for the appropriate conditions for analysis of the gluten macopolymer's (GMP) structure in wheat based foods such as bread, pasta or noodles. The literature search compiled recent or highly cited papers that undertook CLSM using Rhodamine B and/or fluorescein isothiocyanate dyes (FITC). The Table below displays the sample preparation method described in each paper. NA, indicates where information on preparative step was lacking. A variety of preparative techniques were used to examine the structure of the GMP in wheat-based products.

Sample	Dye addition	FITC conc	Rhodamine conc	Dye ratio	FITC dissolution	Rhodamine dissolution	Staining times	Wash step	Sample
Martins et al. 2017 (Martins et al., 2017)	Dough	Coverslip	NA	0.002 g/100 mL		NA	Water	NA	NA
Corral et al. 2017 (Corral et al., 2017)	Dough	Added to dough	0.0010%	0.0001%	10:1	Water	Water	1 hour in dark	Rinsed then coverslipped
Bigne et al. 2017 (Bigne et al., 2017)	Dough	Added to dough	0.0100%	0.0010%	10:1	Water	Water	1 hour in dark	Rinsed then coverslipped
Sciarini et al. 2011 (Sciarini et al., 2012)	Flour	Added to dough	1.0000%	0.1000%	10:1	Water	Water	NA	NA

Wang et al. 2016 (Wang et al., 2016)	Noodles	Cyrotome	0.0250%	0.0250%	1:1	Water	Water	NA	NA
Salgado-Cruz et al. 2017 (Salgado-Cruz et al., 2017)	Pita bread	Heparin wash	0.8500%	0.1500%	0.85:0.15	Water	Water	NA	NA
Lafarga et al. 2016 (Lafarga et al., 2016)	Bread	Fixed in resin	0.1000%	NA	NA	ethanol	NA	Added 1 drop	Rinsed, placed on glass slide
McCann & Day 2013 (McCann & Day, 2013)	Bread	During mixing	0.0100%	0.0100%	1:1	Water	Water	NA	NA

Minarro et al. 2012 (Miñarro et al., 2012)	Bread	Cut and dropped into bread	NA	10 ⁻⁹ %	NA	NA	Water	NA	NA
Schober et al. 2007 (Schober et al., 2007)	Bread	Cut and dropped into bread	NA	NA	NA	NA	NA	1 hour in dark	NA
Primo-Martín et al. 2007 (Primo-Martín et al., 2007)	Bread	Added to dough	0.8500%	0.1500%	0.85:0.15	Water	Water	NA	NA
Primo-Martín et al. 2006 (Primo-Martín et al., 2006)	Bread	Added to dough	1.0000%	0.1000%	10:1	dimethylformamide	dimethylformamide	NA	NA

Zou et al. 2005 (Zou et al., 2015)	Pasta	Added to liquid	0.0500%	0.0500%	1:1	Water	Water	30 mins	Rinsed then mounted on water and glass slides
Zheng et al. 2016 (Zheng et al., 2016)	Pasta	Dropped into pasta	1.0000%	0.1000%	10	ethanol	ethanol	NA	NA