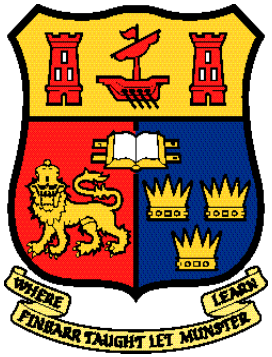
Brewers' spent grain: a potential source of health enhancing ingredients

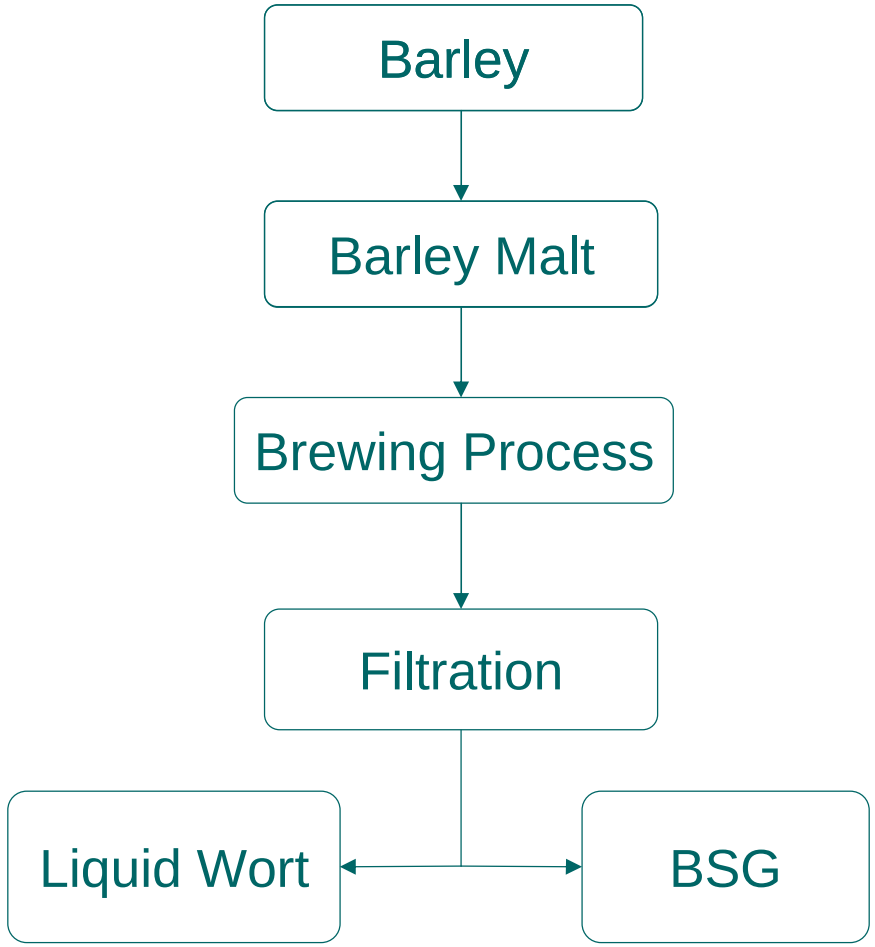
Dr. Charles Piggott & Prof. Dick FitzGerald

Department of Life Sciences,

University of Limerick



Generation of Brewer's Spent Grain (BSG)



Pale BSG

Black BSG

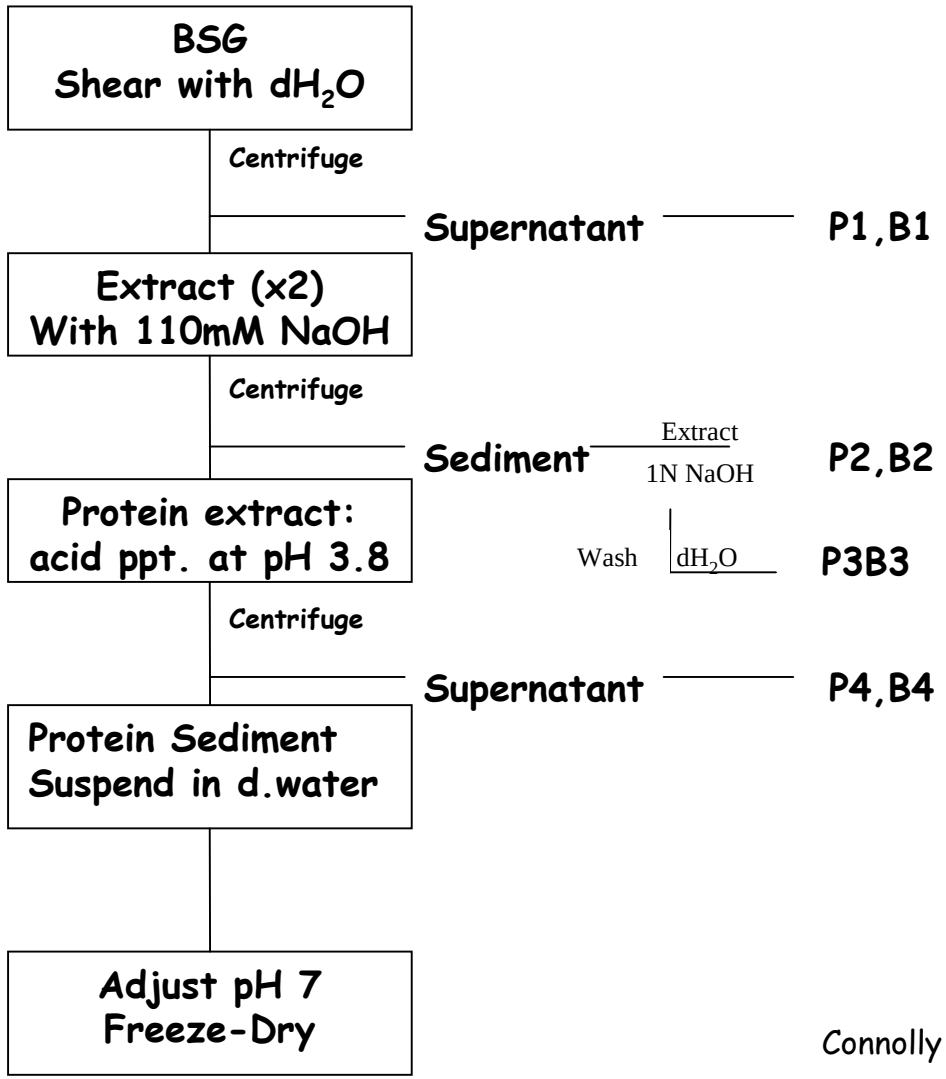
Composition of pale and black brewers' spent grain (BSG)

Component	Pale	Black
	(mg/100mg BSG dw)	
Protein	23.10 ± 0.09	26.93 ± 0.69
Lipid	13.51 ± 0.78	9.96 ± 0.09
Lignin (incl. hemicellulose)	23.39 ± 0.56	n.d.
Non-starch carbohydrate	34	n.d.
Starch	1.48 ± 0.01	0.85 ± 0.02
Polyphenol	1.70 ± 0.01	2.61 ± 0.07
Ash	3.29 ± 0.06	2.07 ± 0.03

n=2

Connolly et al., 2013

Scheme for extraction of protein-rich isolates from pale and black BSG

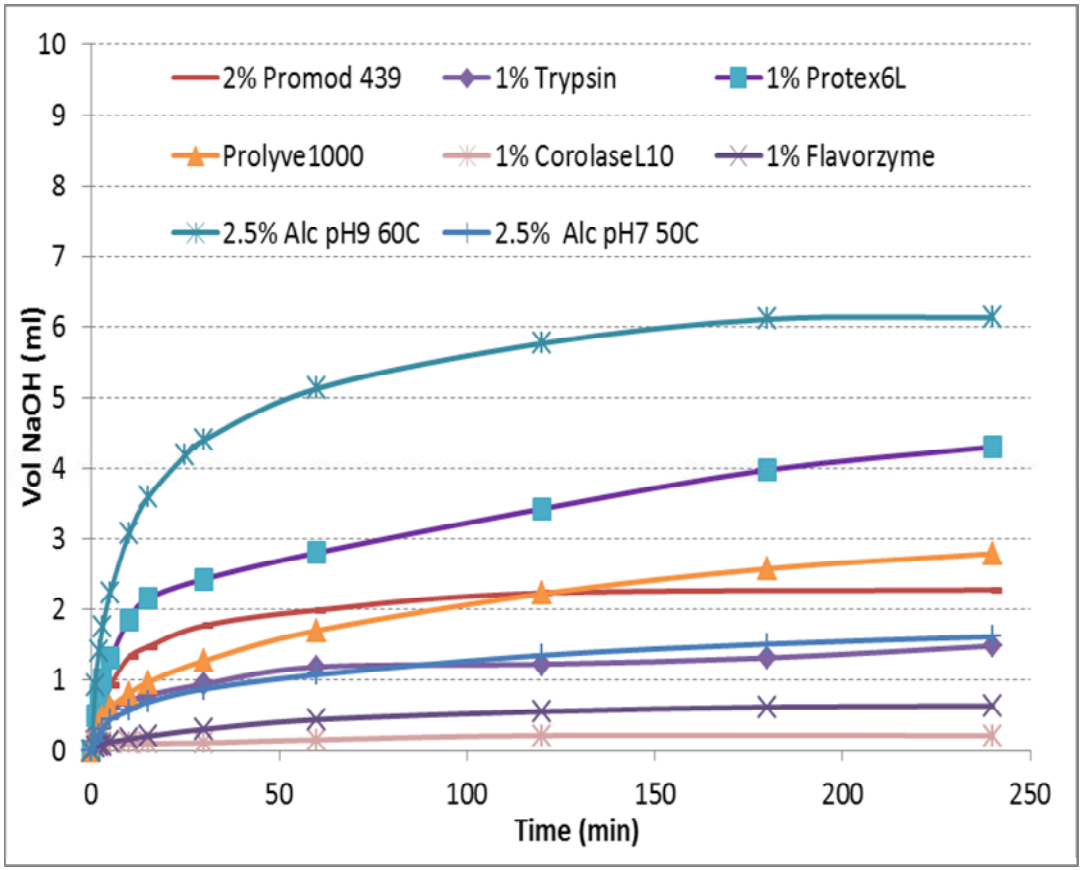


Connolly et al., 2013

Bioactivity assessment of BSG ingredients (*in vitro*)

- Antioxidant activity using FRAP, DPPH and TBARS assays
- Antioxidant activity (DNA damage) using Comet assay
- Anti-inflammatory activity (cytokine production) ELIZA assay
- Anti-diabetic activity (α-glucosidase/α-amylase inhibition)
- Anti-diabetic activity (DPP-4 inhibition)
- Blood pressure control (ACE inhibition)

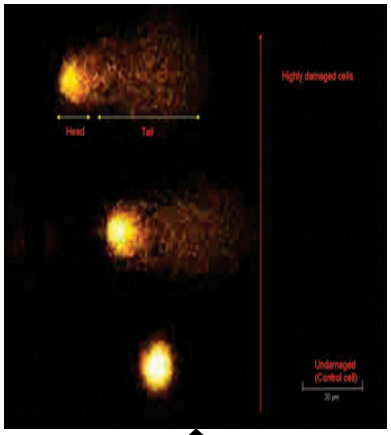
Enzymatic hydrolysis pale BSG protein isolate.



Connolly et al., 2013

DNA protective effects - Comet assay

- To evaluate oxidative DNA damage in cells treated with phenolic extracts (2.5% v/v), ferulic acid (1μg/ml) or protein hydrolysates (0.5% v/v)



Cells
Cells exposed to oxidant following incubation for 24hrs with sample/standard

Slides
LMP agarose

Lysis
60mins @ 4°C

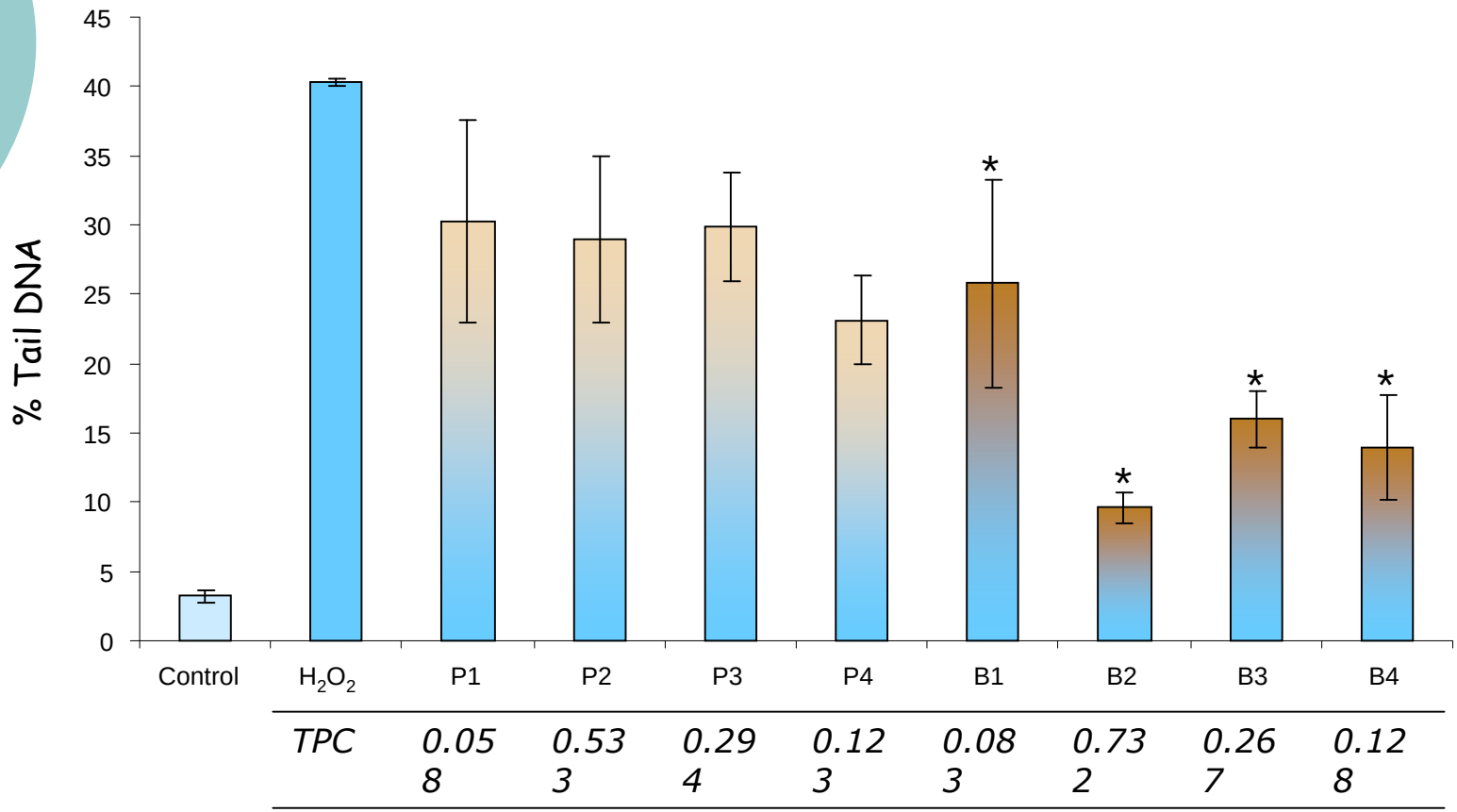
Electrophoresis
•25mins @ 21V / 300mA
•negatively charged DNA migrates toward anode

Neutralisation
with TRIS 5mins X 3

Staining
with EtBr 5mins

Scoring
fluoresence 510-550 nm and Komet 5.5 image analysis software

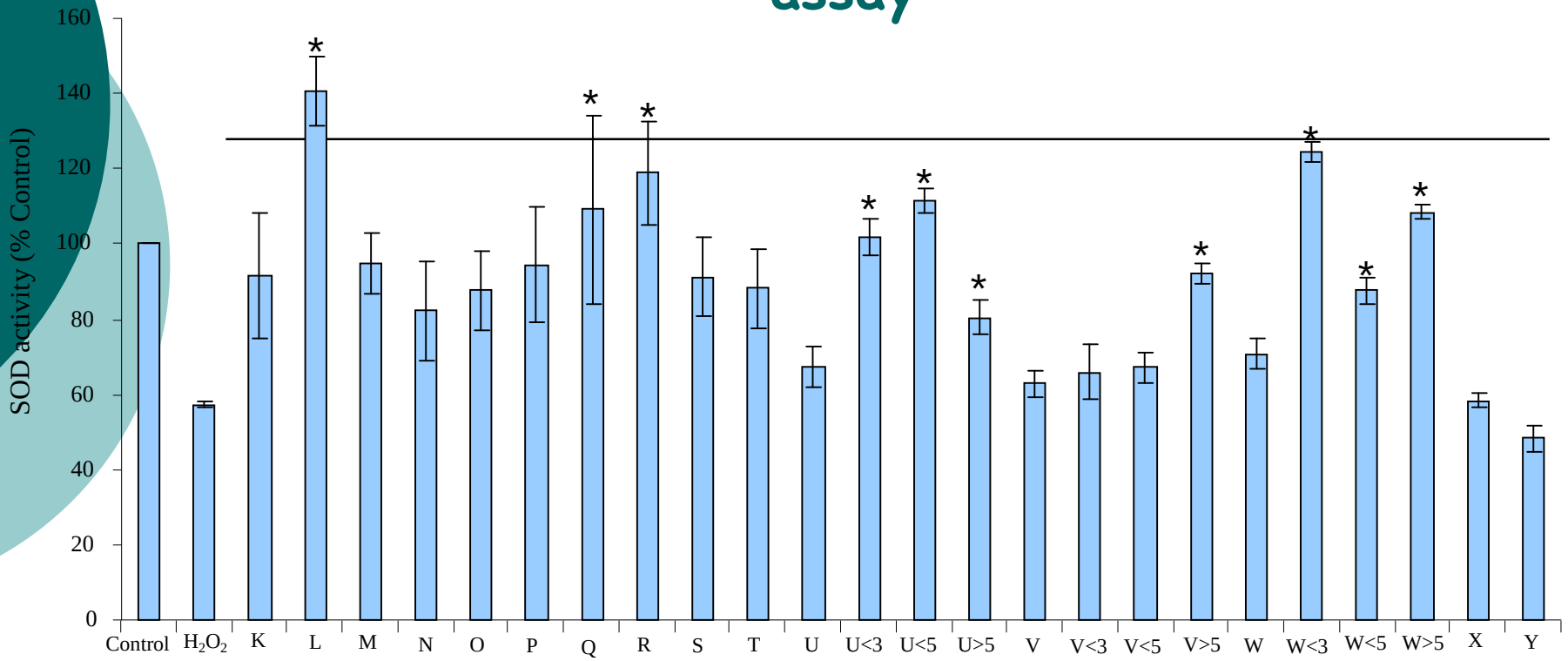
BSG phenolic extracts - DNA damage in U937 cells following exposure to H_2O_2 – Comet assay



n=4 individual experiments, * significantly different from H_2O_2-treated cells, ANOVA followed by Dunnetts

McCarthy et al., 2012

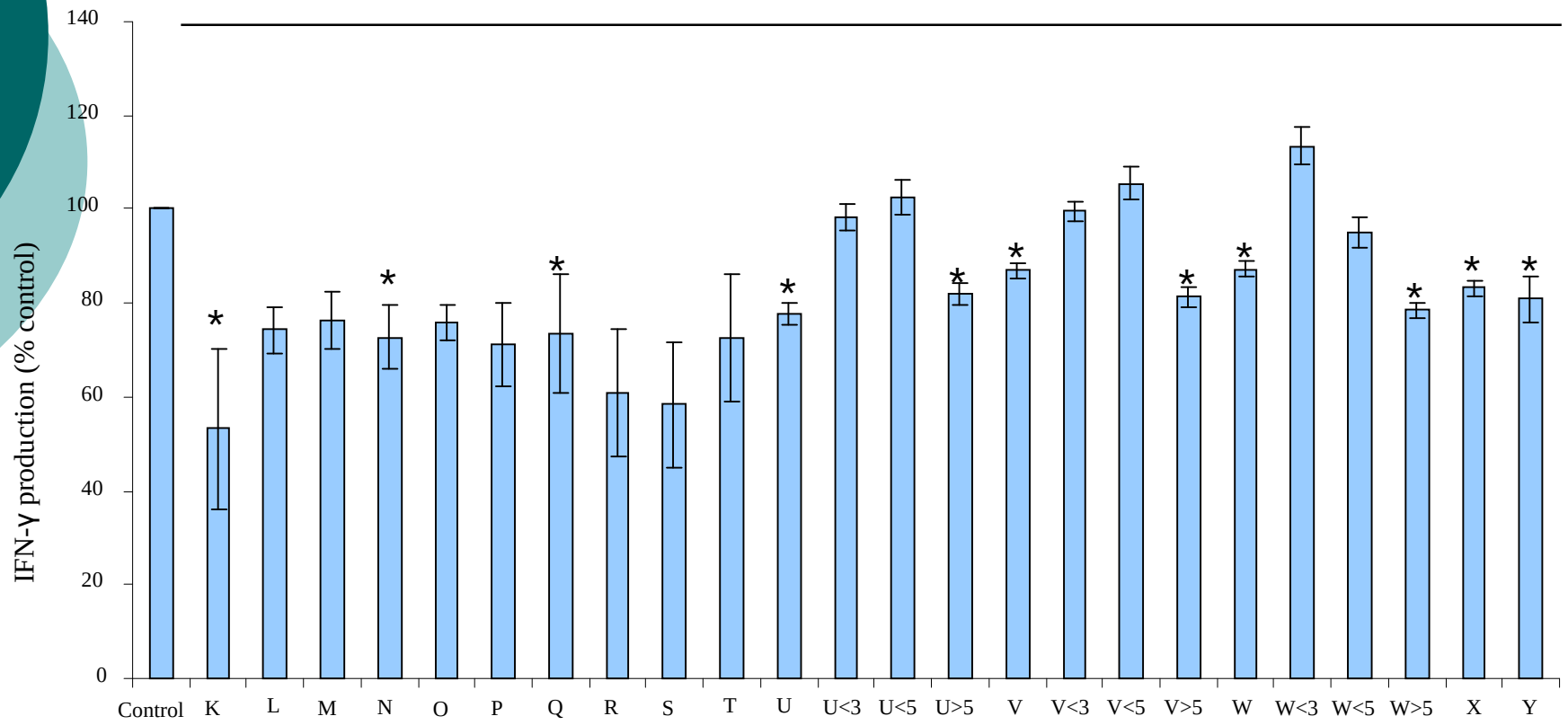
BSG protein hydrolysates – antioxidant effect in U937 cells following exposure to H_2O_2 – SOD assay



Data represent the mean ± s.e. of four independent experiments. Statistical analysis by ANOVA followed by Dunnett's test. * Denotes significant difference in SOD activity between H_2O_2 control and protein hydrolysate (P<0.01)

McCarthy et al., 2012

Immunomodulatory effects of BSG protein hydrolysates in con-A stimulated Jurkat T cells

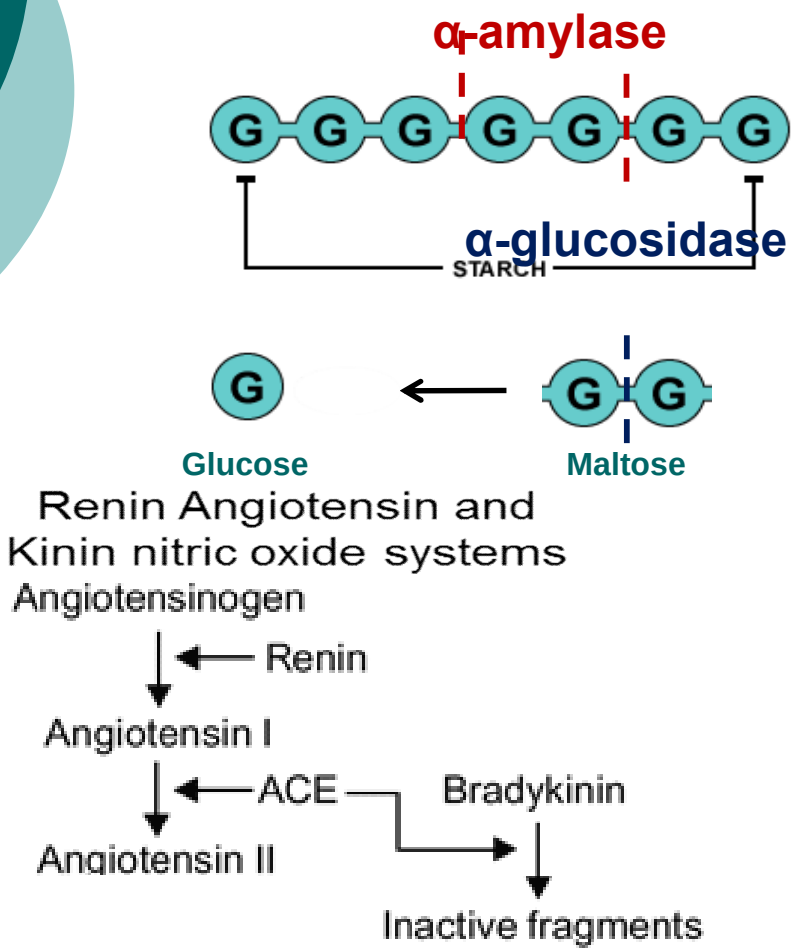


Data represent the mean ± s.e. of three independent experiments. Statistical analysis by ANOVA followed by Tukey's multiple comparison test. * Denotes significant reduction in IFN-γ production, relative to con-A treated Jurkat T cells control (P<0.05)

McCarthy et al., 2013

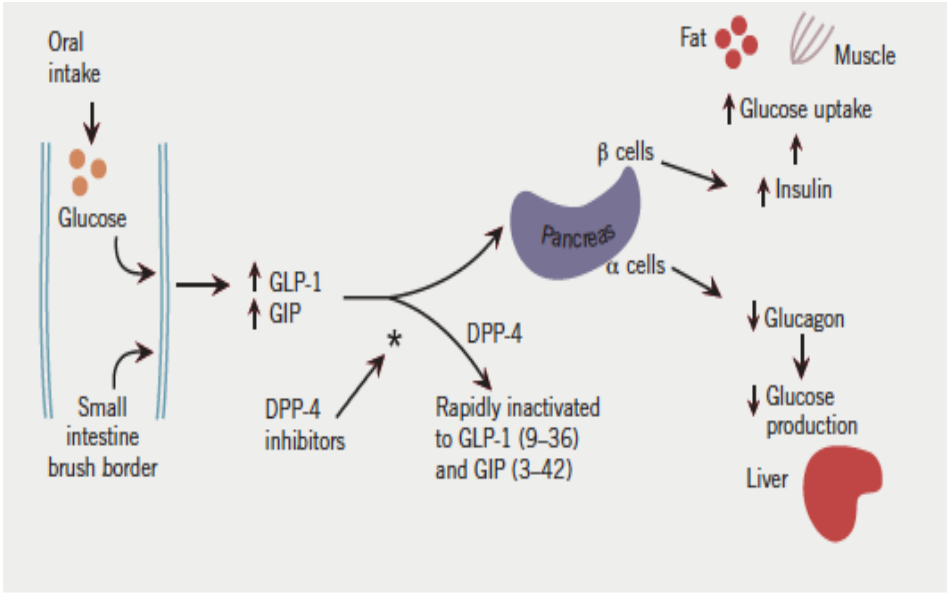
Inhibition of α-amylase, α-glucosidase, dipeptidyl peptidase-IV (DPP-IV) and angiotensin converting enzyme (ACE)

Breakdown of starch



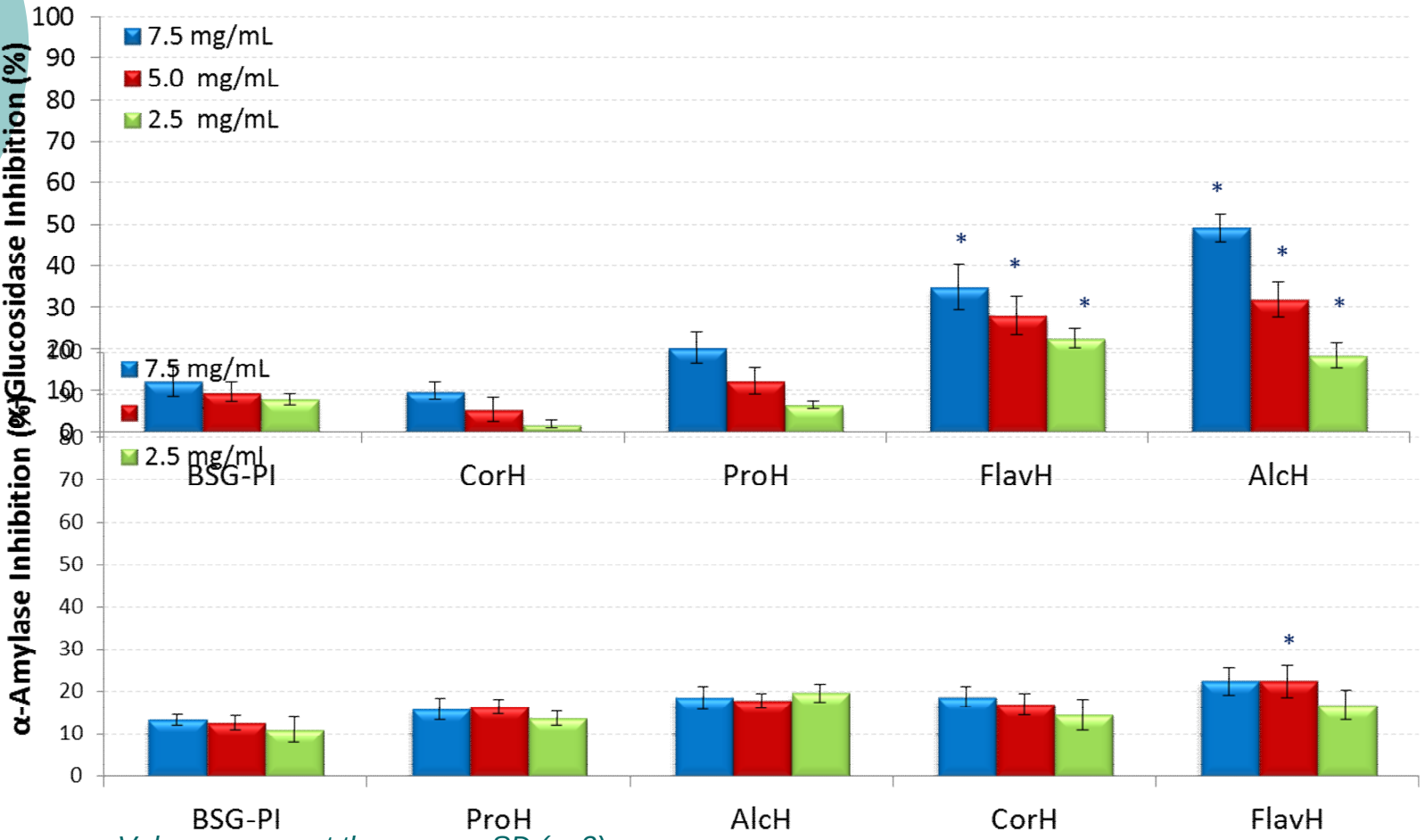
Adapted from FitzGerald et al. (2004)

DPP-IV inactivation of incretin hormones GIP and GLP-1



Taken from McDougall et al. (2011)

α-Glucosidase and α-amylase inhibition of pale BSG-PI and associated hydrolysates

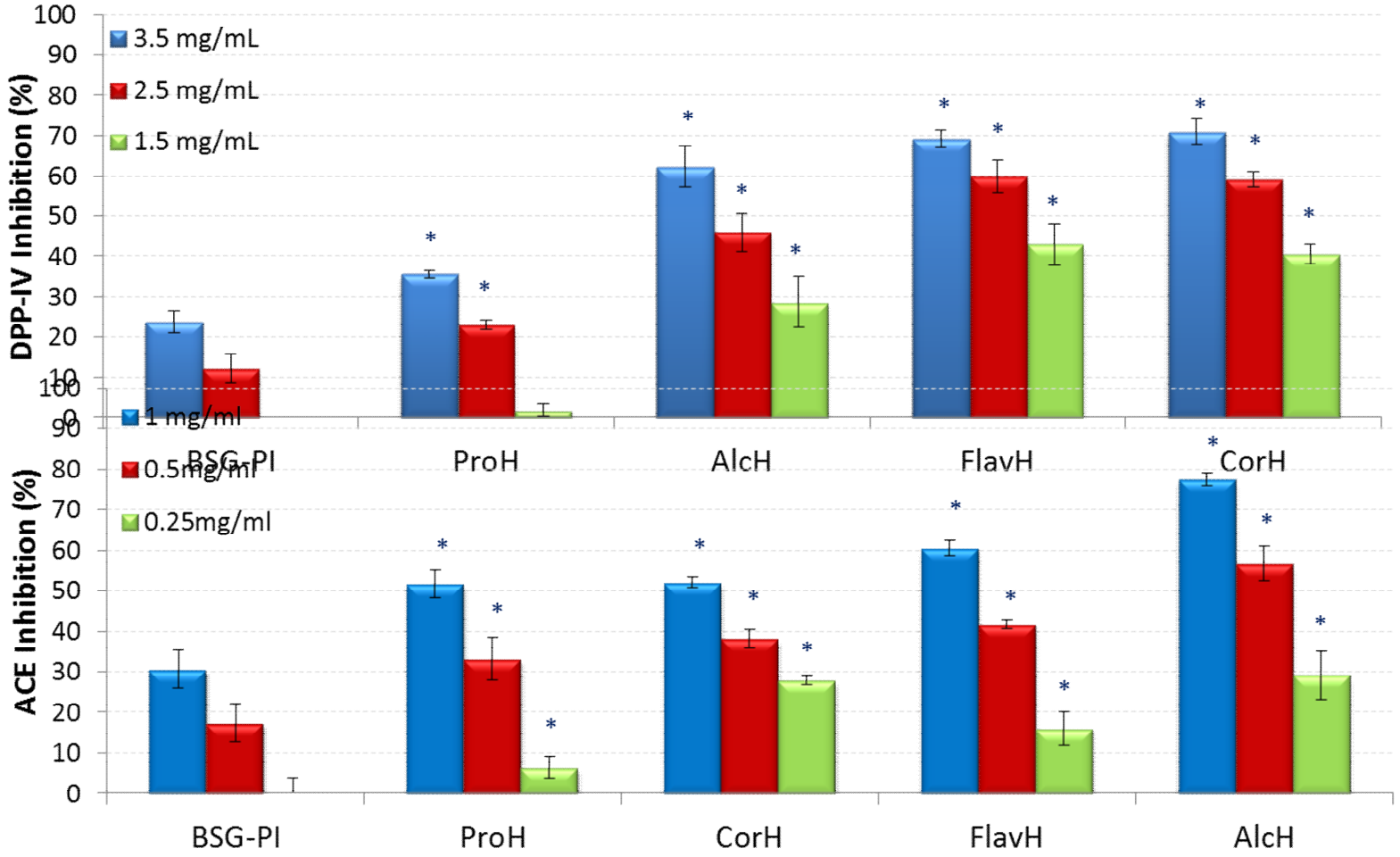


Values represent the mean ± SD (n=3).

Asterix denotes samples were significantly different to the corresponding control ($p<0.05$)

Connolly et al., 2013 (submitted)

DPP-IV and ACE inhibitory activity of pale BSG-PI and associated hydrolysates

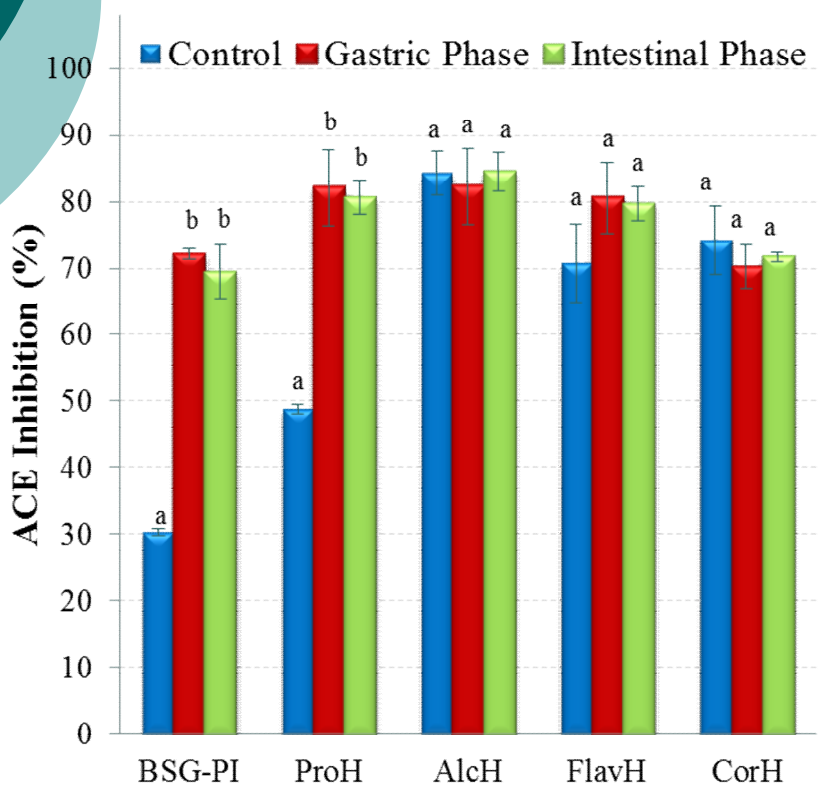


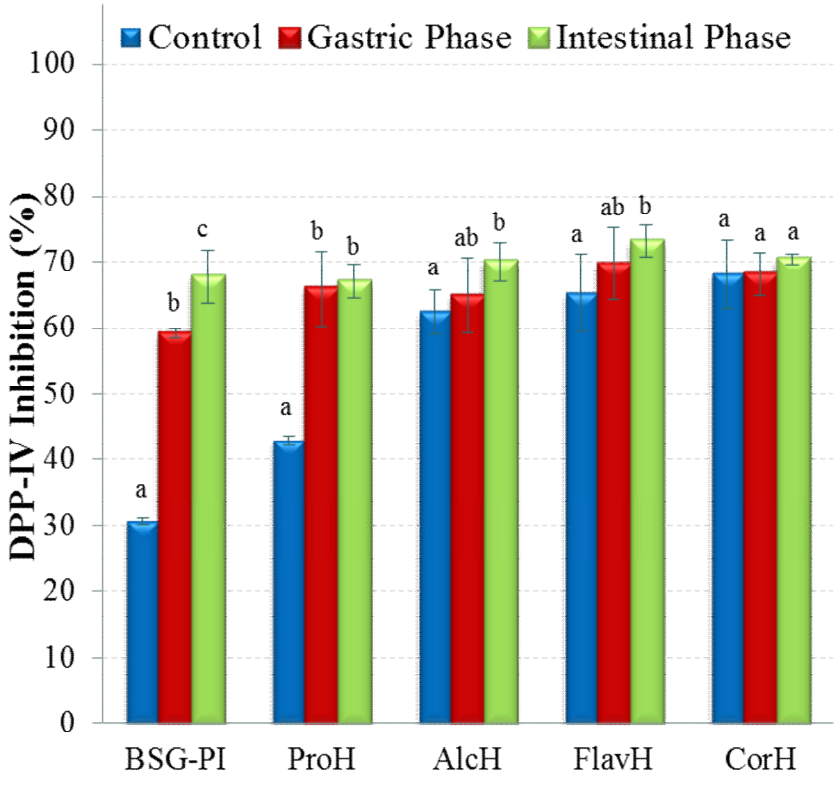
Values represent the mean ± SD (n=3).

Asterix denotes samples were significantly different to the corresponding control ($p<0.05$) Connolly et al., 2013 (submitted)

DPP-IV and ACE inhibitory activity of pale BSG-PI and associated hydrolysates after simulated gastro intestinal digestion

Gastric phase: 1% pepsin at pH2.0 for 90min
Intestinal phase: 2.5% Corolase PP At pH 7.5 for 150 min
(Walsh et al., 2004)





Values represent the mean ± SD (n=3).
Samples with different letters in the same sample set were found to be significantly different ($p<0.05$)

Connolly et al., 2013 (submitted)

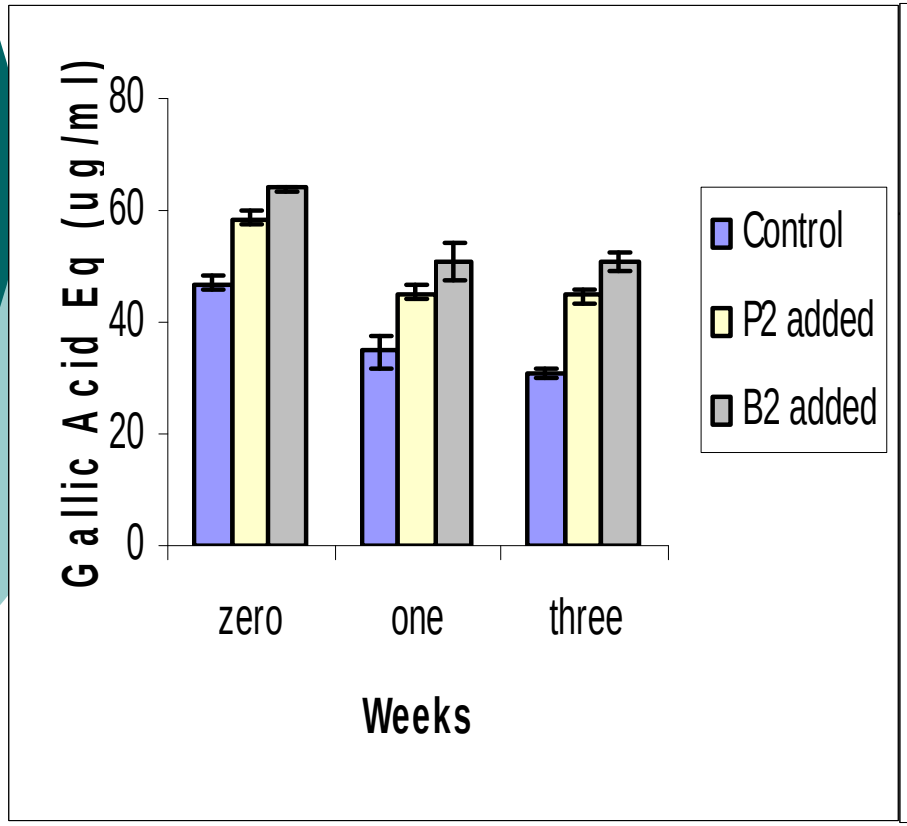
Food fortification with bioactive BSG fractions

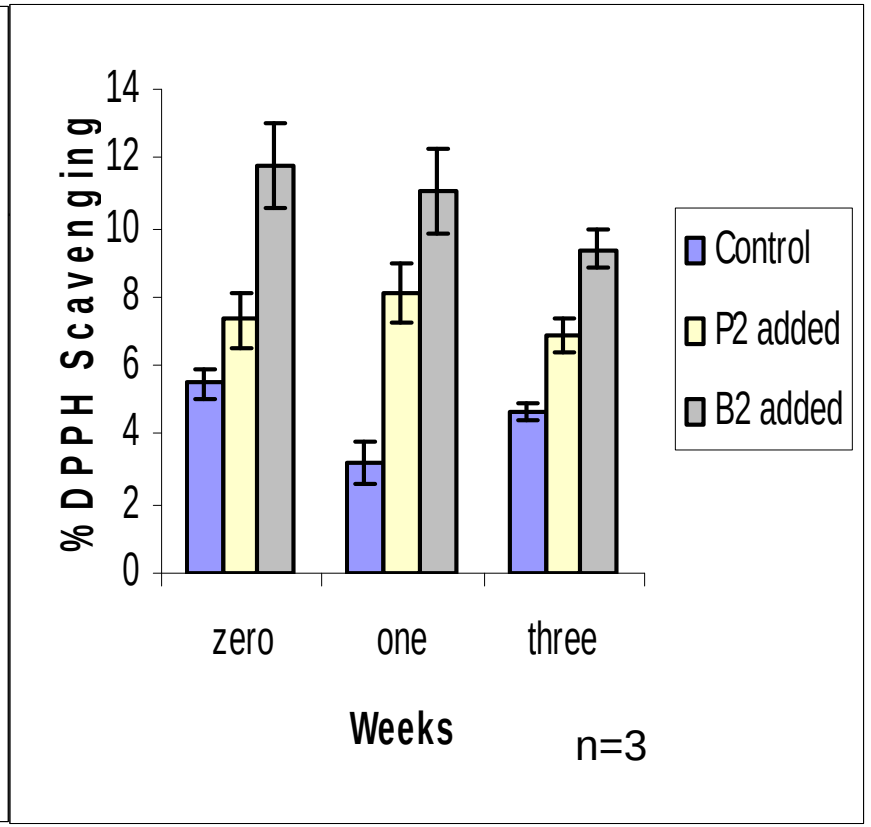
- Identification of BSG fractions/ingredients suitable for incorporation into formulated foods
- Fortification of meat, juices, chocolate drink, snack-bar and yogurt with bioactive BSG extracts
- Study of bioactivity retension within the food systems using standard bioactivity assays and simulated gastro-intestinal digestion (SCID)

Polyphenol levels in co-product fractions post protein extraction from pale and black brewers' spent grain (BSG)

Fraction	Polyphenol (mg GAE/g BSGdw)	
	Protein extraction (20°C)	Protein extraction (50°C)
P1	0.15 ± 0.01	0.25 ± 0.01
P2	3.88 ± 0.09	3.75 ± 0.12
P3	1.32 ± 0.02	0.99 ± 0.03
P4	1.31 ± 0.02	4.59 ± 0.11
Total	6.66	9.58
B1	0.97 ± 0.11	1.48 ± 0.03
B2	6.81 ± 0.14	5.29 ± 0.05
B3	1.99 ± 0.07	1.38 ± 0.03
B4	2.75 ± 0.07	4.35 ± 0.08
Total	12.52	12.50 (n=3)

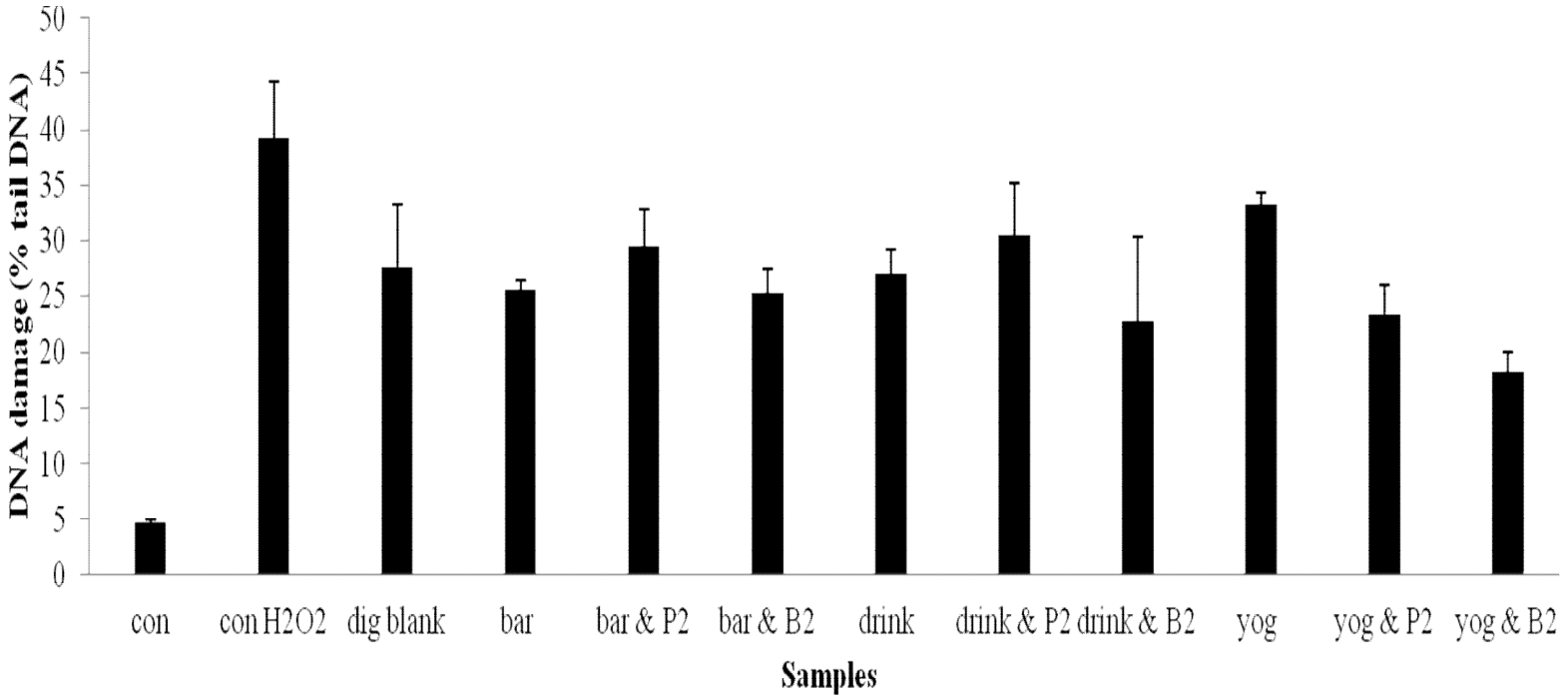
Polyphenol and antioxidant (%DPPHsc) levels in snack-bar matrix fortified with BSG phenolic fractions P2 and B2





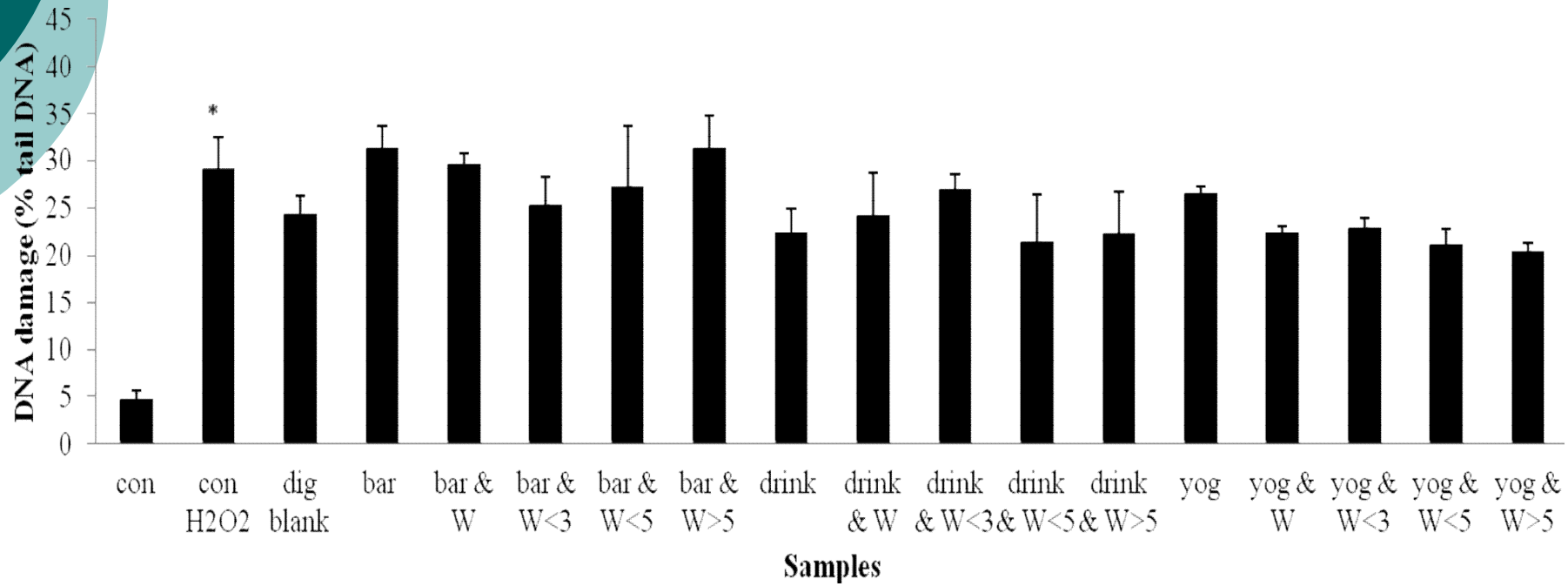
Fortified with P2 and B2 2.33 % (w/w): stored at room temperature (in dark) until analysis

Protection by BSG phenolic extract-fortified food digestates against H_2O_2 induced DNA damage in Caco-2 cells



McCarthy et al., 2013 (submitted)

Protection by BSG protein hydrolysate-fortified food digestates against H_2O_2 induced DNA damage in Caco-2 cells



McCarthy et al., 2013 (submitted)

Summary - potential applications of brewers' spent grain bioactive extracts

- Extraction and isolation procedures optimised for protein-rich isolates, protein hydrolysates and phenolic-rich fractions from BSG.
- BSG extracts possess antioxidant properties (FRAP, DPPH, TBARS and protection against oxidant induced DNA damage), exhibit α-glucosidase, DPP-4 and ACE inhibition and the ability to decrease cytokine production.
- BSG extracts may have potential health benefits, particularly in relation to conditions such as oxidative stress, hypertension, inflammatory disease and type 2 diabetes.
- BSG extracts have potential applications in formulated foods, providing a novel source of biofunctional ingredient.
- Future work involves identification of bioactive peptides using LC-MS/MS, and *in vivo* animal studies followed by *in vivo* human intervention studies are needed to assess whether the bioactivity is retained.

Peer reviewed publications

1. L. McCarthy, Y. C. O'Callaghan, A. Connolly, C. O. Piggott, R. J. FitzGerald, N. M. O'Brien. (2012) Phenolic extracts of brewers' spent grain (BSG) as functional ingredients – Assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. *Food Chemistry*, (134) 641-646.

2. Connolly A., Piggott C.O. and FitzGerald R.J. (2013) Characterisation of protein rich isolate and antioxidative phenolic extracts from pale and black brewers' spent grain. *International Journal of Food Science and Technology*. 48, 1670-1681.

3. McCarthy, A.L., O'Callaghan, Y.C., Piggott, C.O., FitzGerald, R.J., O'Brien, N.M. (2013) Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review. *Proceedings of the Nutrition Society*, 72, 117-125.

4. McCarthy, A.L., O'Callaghan, Y.C., Connolly A, Piggott, C.O., FitzGerald, R.J., O'Brien, N.M. (2013) In vitro antioxidant and anti-inflammatory effects of brewers' spent grain protein rich isolate and its associated hydrolysates. *Food Research International*, 50, 205-212

5. McCarthy, A.L., O'Callaghan, Y.C., Connolly A, Piggott, C.O., FitzGerald, R.J., O'Brien, N.M. (2013) Brewers, spent grain (BSG) protein hydrolysates decrease hydrogen peroxide (H_2O_2)-induced oxidative stress and concanavalin-A (con-A) stimulated IFN-γ production in cell culture. *Food & Function*, 4, 1709-1716.

6. McCarthy, A.L., O'Callaghan, Y.C., Neugart, S., Piggott, C.O., Connolly, A., Jansen, M.A.K., Krumbein, A., Schreiner, M., FitzGerald, R.J., O'Brien, N.M. (2013) The hydroxycinnamic acid content of barley and brewers' spent grain (BSG) and the potential to incorporate phenolic extracts of BSG as antioxidants into fruit beverages. *Food Chemistry*, 141, 2567-2574.

Acknowledgments

Team members at UL: Prof. Dick FitzGerald, Dr. Charles Piggott and Dr. Alan Connolly

Team members at UCC: Prof Nora O' Brien, Dr. Yvonne O' Callaghan and Dr. Aoife McCarthy

Funding from the DAFF FIRM Programme 08RDUL699

THE DEPARTMENT OF
AGRICULTURE, FISHERIES & FOOD
AN ROINN TALMHAÍOCHTA, IASCAIGH & BIA