GUT FERMENTATION PRODUCTS OF WHEAT ALEURONE SUPPRESS CELL GROWTH AND SURVIVAL OF HUMAN ADENOCARCINOMA CELLS

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Background

Wheat grain and particularly the aleurone layer (Fig. 1) contain high amounts of dietary fibre. Fermentation of dietary fibre by human gut flora may enhance level of short-chain fatty acids (SCFA) which are potentially chemoprotective e.g. by suppressing the growth of tumour cells. Furthermore lower amounts of potentially tumour promoting products such as deoxycholic acid (DCA), ammonia and hydrogen peroxide (H_2O_2) may be produced.

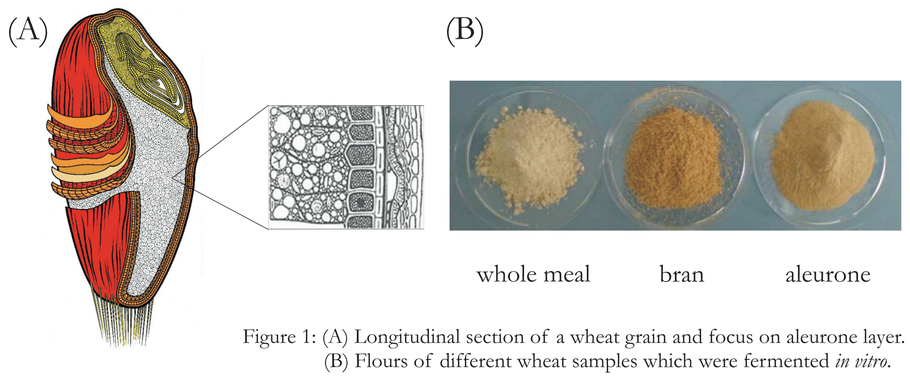

Figure 1: (A) Longitudinal section of a wheat grain and focus on aleurone layer. (B) Flours of different wheat samples which were fermented *in vitro*.

Methods

Wheat aleurone, whole meal wheat flour and wheat bran were digested and fermented *in vitro*. Fermentation supernatants (fs) were analysed for SCFA, DCA, ammonia and H_2O_2. Corresponding mixtures of SCFA and the individual substance butyrate were prepared. HT29 adenocarcinoma cells were treated for 24-72 h with butyrate, mixtures or complex fs. Cell survival was determined by quantifying fluorescence of DAPI-labelled DNA.

Results

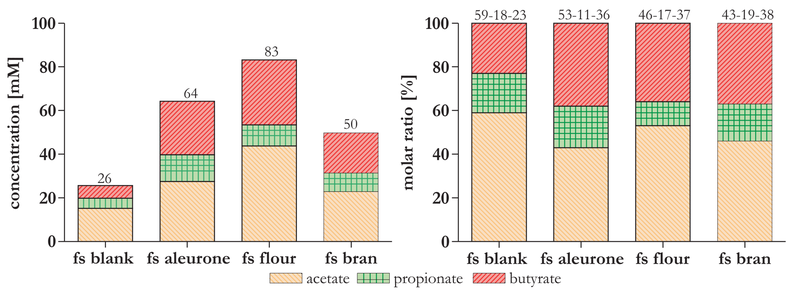

Figure 2: Concentrations and molar ratios of SCFA in complex fs of wheat samples and control (blank) analysed by GC-MS.

Fermented wheat samples contained 2-3 fold higher amounts of SCFA than control (Fig. 2, 3).

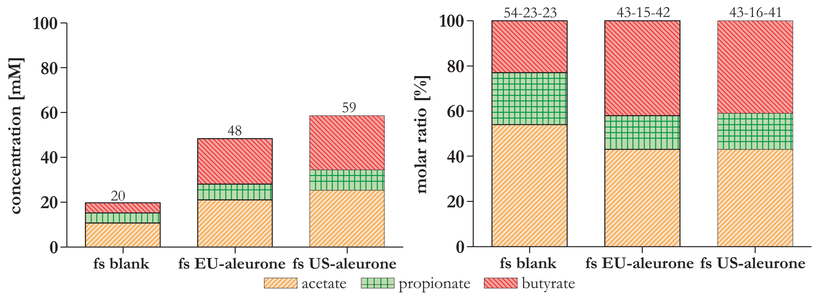

Figure 3: Concentrations and molar ratios of SCFA in complex fs of EU- and US-aleurone and control (blank) analysed by GC-MS.

Table 1: Concentrations of DCA, H_2O_2 and ammonia in complex fs of wheat samples and control (blank) analysed by HPLC-MS/MS, FOX2 assay and Berthelot reaction.

	DCA [µM]	H_2O_2 [µM]	ammonia [mM]
fs blank	35.4	87.4	10.9
fs aleurone	10.3	90.0	18.5
fs flour	2.6	74.7	11.6
fs bran	9.1	83.1	14.6

Fermented wheat samples contained reduced levels of DCA. H_2O_2 production was not influenced whereas more ammonia was produced (Tab. 1, 2).

Table 2: Concentrations of DCA, H_2O_2 and ammonia in complex fs of EU- and US-aleurone and control (blank) analysed by HPLC-MS/MS, FOX2 assay and Berthelot reaction.

	DCA [µM]	H_2O_2 [µM]	ammonia [mM]
fs blank	46.65	77.6	11.9
fs EU-aleurone	5.04	100.6	16.6
fs US-aleurone	5.93	106.5	21.3

Fermented wheat samples suppressed growth of tumour cells. Fs inhibited cell growth more than synthetic mixtures. Growth inhibitory activity of SCFA was mainly caused by butyrate (Fig. 4).

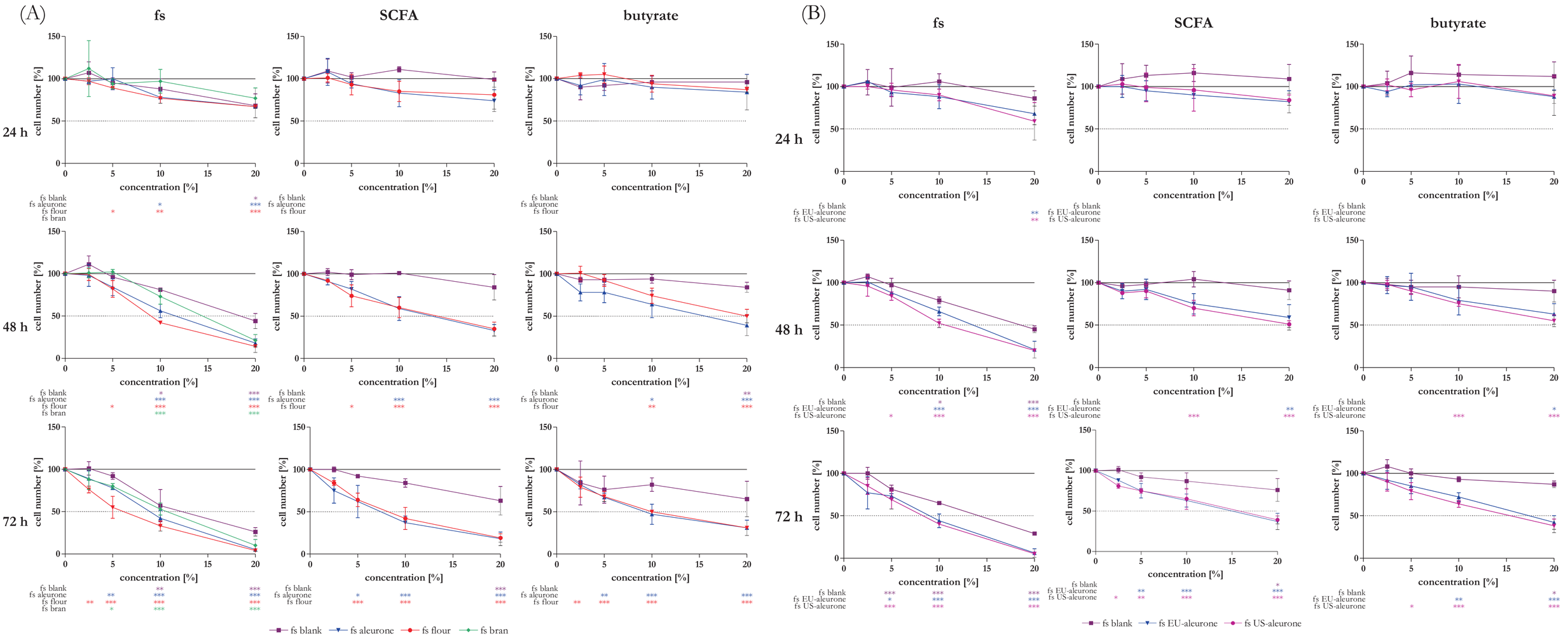

Figure 4: Inhibition of cell growth after incubation of HT29 cells with complex fs and corresponding synthetic mixtures of (A) different wheat samples or (B) two wheat aleurone varieties (EU: european, US: american) and control (blank). Shown are means ± SD (n=3) and significant differences of respective concentrations of fs or synthetic substances to medium control ($p<0.05$: *; $p<0.01$: **; $p<0.001$: ***).

Conclusions

Gut flora-mediated fermentation of wheat aleurone independently from variety results in reduced level of tumour promoting DCA and higher levels of SCFA especially butyrate, which inhibits growth of human adenocarcinoma cells. The fact that complex fs were more cytotoxic than corresponding synthetic mixtures of SCFA and butyrate point to the involvement of additional growth inhibitory effects of other bacterial metabolites. Most of the activities of whole meal and of bran are due to their contents of aleurone.

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