Pea flour digestibility and fermentability measured with $^{13}$C isotopes.

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It is well established that some starches are resistant to human digestive enzymes and enter the large intestine where they may be fermented by the colonic bacterial flora to short chain fatty acids and gases (CO$_2$, H$_2$, CH$_4$) [1].

It is difficult to measure the digestibility and fermentability of starch foods in vivo, in normal man, and most studies have been performed with ileostomy patients or animal studies. Use of breath hydrogen to quantify the malabsorption of carbohydrate is not quantitative as hydrogen production can be influenced by many other factors than the carbohydrate source.

An alternative approach is to use $^{13}$C labelled starchy foods and to measure the $^{13}$C enrichment of breath CO$_2$. This is released by human cells from the metabolism of glucose, indicating small intestine digestion and absorption, or from bacterial fermentation of carbohydrate in the colon. Fermentation may release CO$_2$ directly or indirectly by human metabolism of the short chain fatty acids produced.

Pea plants (Bacarra variety) were grown and as soon as pods began to form were placed in a $^{13}$CO$_2$ enriched environment in polypropylene bags, (Propylene CD 35; ICI Welwyn Garden City UK) approx 154 x 30 x 45 cm to fit individual plants, and sealed air-tight. 250 mls of $^{13}$CO$_2$ (99 atom %, Cambridge Isotope Laboratories, Welwyn Garden Cit y) were added and the bags were then filled to capacity with room air using an air pump. The plants were incubated for 6 days on two occasions separated by 1 week. Peas were allowed to ripen under normal conditions and the peas harvested and dried to form pea flour, enriched by 8.64 atom % $^{13}$C excess.

After an overnight fast, six human subjects ingested 300mg of enriched pea flour contained in 175g biscuits. The digestibility of starch, measured in an in vitro model, was 7.5% rapidly digestible starch, 33.1% slowly digestible starch and 11.4% resistant starch.

Breath samples were taken every 30 mins for up to 24 hours, with a gap when subjects were asleep, and analysed for hydrogen and $^{13}$CO$_2$ enrichment. Subjects were asked to avoid naturally $^{13}$C enriched foods but were allowed to eat a low fat/low fibre lunch and evening meal.

The profiles of enrichment of $^{13}$CO$_2$ in breath showed a complex of three apparent peaks. Each peak was not easily separated by eye for every subject and the second peak usually formed a shoulder on the trailing end of the first peak. However this second peak occurred as the breath hydrogen began to rise indicating the meal had entered the large intestine. Fig 1 shows a typical profile.

The third peak in $^{13}$CO$_2$ enrichment occurred much later between 12 and 20 hours in individual subjects. In some subjects this occurred when they were asleep and so critical information about the height and duration of the peak was lost. The late timing of this peak is consistent with previous reports of breath hydrogen from fermentation of resistant starch [2].

Figure 1 Breath $^{13}$CO$_2$ and H$_2$ after ingestion of biscuits containing 300mg labelled pea flour in one human volunteer.

Mathematical modelling [3] and longer sampling periods, to separate small intestinal and colonic events, should allow this method to quantify digestibility and fermentability of starchy foods in vivo.

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