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In vitro digestion of short-dough biscuits enriched in proteins and/or fibres using a multi-compartmental and dynamic system (2): Protein and starch hydrolyses

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The influence of protein and/or fibre enrichment on the nutritional properties of biscuits was studied in terms of proteolysis and amylolysis. Biscuits were digested using a multi-compartmental and dynamic system that simulates the main physiological digestive functions of the upper tract of healthy adult humans: the TIM-1. A control biscuit and three biscuits enriched in proteins and/or fibres were digested under the same conditions. Samples were collected in each compartment of the TIM-1 (stomach, duodenum, jejunum and ileum) at different times of digestion and analysed in terms of proteolysis and amylolysis. Results indicate that both formulation and processing impacted the digestive fate of the biscuits. Incorporating proteins or fibres in biscuits lowered or delayed proteolysis. Moreover a protein-plus-fibre additional or synergic effect was observed. Biscuits enriched in proteins and/or fibres displayed a higher amylolysis degree than the control biscuit, probably due to lower starch amounts and higher gelatinization degrees.

1. Introduction

In the general context of weight management, the development of food products having a higher satiating power appears as an alternative or complementary answer to calorie-reduced diets or portion-size control. Dietary fibres, especially viscous soluble ones, and proteins can be used to design satiating products. Several studies conducted with humans showed a higher satiating effect of proteins compared to other macronutrients (Anderson & Moore, 2004). Ingesting proteins generates pre- and post-absorptive signals which contribute to controlling the kinetics of gastric emptying, pancreatic secretion and food intake. Those signals are transmitted to the brain through two main pathways (Bowen, Noakes, & Clifton, 2006). A direct transmission occurs via hormones and nutrients, through the blood system. An indirect transmission through the nervous system involving neurotransmitters is also involved.

Dietary fibres, and more especially viscous soluble ones, are believed to increase satiety (Slavin & Green, 2007). Dietary fibres were shown to slow down the postprandial blood glucose response through several mechanisms, including delayed gastric emptying, reduced amylolysis, reduced diffusion of starch hydrolysis products towards the intestinal mucosa and reduced glucose absorption. The influence of fibres on starch digestibility depends on the nature of the fibres (endogenous or exogenous fibres) and on their thickening abilities.

In the case of endogenous fibres, maintaining the botanical tissue as intact as possible by preserving the integrity of the

Abbreviations: BCS, sodium bicarbonate; BCA, ammonium bicarbonate; SAPP, sodium acid pyrophosphate; TIM-1, TNO Intestinal Model; TCA, trichloroacetic acid; C, the control biscuit; P, the proteins-enriched biscuit; F, the fibres-enriched biscuit; P + F, the biscuit enriched in both proteins and fibres.

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structure, at a cellular or tissue level, seems to be an important parameter of starch digestibility (Wursch, Delvedovo, & Koellreutter, 1986). It is the case for whole grain tissues with minimal particle size reduction as starch remains embedded in the surrounding matrix. An intact structure might reduce the rate of gastric emptying by increasing the "solid" phase in the stomach. It may also decrease the substrate's availability by providing an enzymatic barrier, for example by encapsulating starch in a network, that would restrict starch swelling and reduce its physical accessibility to the enzyme (Granfeldt, Hagander, & Bjorck, 1995; Liljeberg, Granfeldt, & Bjorck, 1994).

The influence of exogenous fibres depends mainly on viscosity. Several studies showed that the addition of viscous dietary fibres such as guar gum, pectin or beta-glucans reduced metabolic responses like the postprandial glycaemic response and/or starch hydrolysis (Brennan, Blake, Ellis, & Schofield, 1996; Brennan & Cleary, 2007). However, other studies reported no convincing results (Sels et al., 1992). The lack of effects could be explained by the inability of the food process to maintain the viscous properties of the fibre components, which is the case when polymer hydrolysis occurs during processing (Andersson et al., 2004). Maintaining the viscous properties of fibres in the food product seems to be essential to influence amylolysis and/or the metabolic responses. Adding soluble thickening fibres would likely limit starch digestibility and/or metabolic responses by reducing:

(a) the kinetics of enzymatic reactions (Uribe & Sampedro, 2003)
(b) enzyme accessibility to the substrate, by creating a physical barrier around starch granules (Brennan et al., 1996; Cleary, Andersson, & Brennan, 2007; Koh, Kasapis, Lim, & Foo, 2009)
(c) the kinetics of gastric emptying (Meyer, Gu, Jehn, & Taylor, 1988) and/or intestinal motility
(d) the diffusion of hydrolysed starch to the small intestine mucosa, thus delaying glucose absorption.

Many *in vitro* studies were conducted to understand the effects of enrichment in fibres or proteins on the nutritional properties of cereal products such as bread, biscuits or pasta (Aparicio-Saguilan et al., 2007; Bilgicli, Ibanoglu, & Herken, 2007; Brennan et al., 1996; Brennan & Cleary, 2007; Sozer, Cicerelli, Heinio, & Poutanen, 2014; Symons & Brennan, 2004). However, very few studies were conducted to understand the effects of a combined enrichment in both proteins and fibres.

Most *in vitro* systems present a limited number of simulated parameters, a compartmental fragmentation and a lack of dynamism. Very few studies were carried out with a system simulating more closely the physiological conditions of digestion such as gastric and/or intestinal emptying, pH and temperature regulation, peristaltic movements, gastrointestinal secretions (digestive enzymes, mineral solutions, and acid or base solutions), transit time, water and digestion products absorption.

The TNO gastro-Intestinal tract model (TIM-1) is a multi-compartmental and dynamic computer-controlled system that simulates the main physiological digestive functions of the stomach and small intestine. It was developed by the Department of Physiology, TNO Nutrition and Food Research Institute (Zeist, The Netherlands). The TIM-1 is certainly one of the most complete and dynamic artificial digestive systems there is. It was selected in this study, to realistically simulate the physiological conditions of digestion in the upper tract of healthy adult humans (Minekus, Marteau, Havenaar, & Huisintveld, 1995, chap. 6).

The objective of this study was to investigate the effects of the enrichment of biscuits in viscous soluble fibres and proteins on starch and protein hydrolyses during the physiological digestive process. Enrichment in proteins on the one hand, and in viscous soluble fibres on the other hand were compared with an enrichment combining both proteins and fibres in order to identify synergies or antagonisms.

2. Materials and methods

2.1. Biscuits composition

The macronutrient compositions of the biscuits are shown in Table 1. Sucrose, canola oil, wheat flour, salt, leavening powders such as sodium bicarbonate (BCS), ammonium bicarbonate (BCA) and sodium acid pyrophosphate (SAPP), and an emulsifier, soy lecithin were used to produce the biscuits. All products had low fat (15%, w/w) and low sugar (15%, w/w) contents. The process was described in a previous work (Villemejane, Roussel, Berland, Aymard, & Michon, 2013). Four biscuits were digested: a control (C), a protein-enriched (P), a fibre-enriched (F) and a protein-and-fibre-enriched biscuit (P + F). They contained, respectively 9%, 23%, 10% and 23% (w/w) of proteins and 5%, 4%, 12% and 12% (w/w) of total dietary fibres (TDF), as mentioned in Table 1. Enrichments were done by replacing flour with pea proteins concentrate (Nutralys F85M, Roquette), whey proteins concentrate (Prolacta 80, Lactalis) and/or oat bran (OatWell 22, DSM). Oat bran contained, on a wet basis, 44.0% of total fibres (with 21.6% of soluble fibres (beta-glucans with a molecular weight of 1500 kg/mol) and 22.4% of insoluble fibres), 24.4%, of carbohydrates (with sugars content below 1.5%), 4.0% of lipids, 20.0% of proteins and 5.3% of water. The rest was ash. Pea proteins concentrate and whey proteins concentrate contained, on a wet basis, respectively 4.0% and 17.0% of carbohydrates, 7.0% and 0.4% of lipids, 81.0% and 75.0% of proteins and 7.0% and 6.0% of water. The rest was ash.

The biscuit enriched in fibres (F) contained slightly more proteins than the control one (C) due to a higher protein content in oat bran than in flour (10% of total proteins for the biscuit enriched in fibres *vs.* 9% for the control one).

Table 1
Composition and thermal properties (T_0, T_p and ΔH/g of starch) of biscuits enriched in proteins and/or fibres.

Biscuit	% Total proteins (w/w)	% Total fibres (w/w)	% Soluble fibres (w/w)	% Starch (w/w)	T_0* (°C)	T_p** (°C)	ΔH/g of starch *** (J/g)	GD**** % gelatinized starch (w/w)	Added water /starch ratio (in dough)
Control	9	5	2	53	59.6	66.9	4.3	47	0.3
Protein	23	4	1.7	38	60.4	67.6	3.8	53	0.5
Fibre	10	12	5.9	42	60.3	67.8	3.8	53	0.6
Protein + fibre	23	12	5.8	29	61.5	68.5	1.4	83	1.5

* T_0: gelatinization onset temperature (difference between the two values below 0.3 °C).
** T_p: gelatinization peak temperature (difference between the two values below 0.3 °C).
*** ΔH/g of starch: enthalpy of gelatinization relative to dry starch (difference between the two values below 0.15 J/g).
**** GD: gelatinization degree (see Eq. (1)).