



## Sourdough bread: Starch digestibility and postprandial glycemic response

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### ABSTRACT

To evaluate the influence of sourdough fermentation on starch digestibility in bread, four experimental breads were obtained, prepared from two different wheat flours (whole or white) by two different leavening techniques (sourdough and with *Saccharomyces cerevisiae*). Products were analyzed for their starch, fiber and resistant starch (RS) content and then submitted to *in vitro* hydrolysis with porcine alpha-amylase. On the same breads, postprandial blood glucose was evaluated in healthy human subjects. Both sourdough fermented breads gave glycaemic responses significantly lower ( $p < 0.001$ ) than the corresponding products leavened with *S. cerevisiae*. On the contrary, the presence of fiber did not influence the glycaemic potential of breads. RS levels were higher in the sourdough products, whereas no differences were observed either in the rate of starch hydrolysis or in the degree of polymerization of the starch residues after the *in vitro* hydrolysis. We may conclude that sourdough fermentation is a technique able to reduce the glycaemic response to bread and that the mechanism does not seem related to the rate of starch hydrolysis.

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The estimated annual intake of bread in European countries has been reported to range from 46 kg (Sweden, Great Britain, Finland and Austria) to 100 kg (Greece, Portugal, Spain and Italy) per person. Starch in bread is rapidly digested and absorbed, producing high glycemic responses (Jenkins *et al.*, 1988). Hyperglycemia is a key factor involved in the etiology of diseases related to the metabolic syndrome, its control being an important nutritional goal. Sourdough fermentation has been reported to improve nutritional properties of starch (Liljeberg *et al.*, 1995). The effect could be attributed to organic acids produced during sourdough fermentation which could ameliorate glucose disposal delaying gastric emptying or suppressing enzymatic activity. The beneficial effect of sourdough fermentation on glycemia has been already proposed by Liljeberg and Bjorck (1994) and Jenkins *et al.* (1986), but in both studies it was impossible to discern between the effect of sourdough fermentation and other factors also able to potentially influence glucose response, such as dietary fiber and starch accessibility. The aim of the present study was to evaluate the possible influence of sourdough fermentation in wholemeal or white breads on starch digestibility and postprandial glycemia in healthy subjects.

*Abbreviations:* BMI, Body mass index; IAUC, Incremental area under the curve; RS, Resistant starch; TS, Total starch.

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Four experimental breads, prepared from two different wheat flours (wholemeal or white) by two different leavening techniques (sourdough or with *Saccharomyces cerevisiae*) were tested for *in vitro* and *in vivo* analyses.

All the breads were analyzed for moisture, ash, protein, fat, total starch (K-TSTA kit, Megazyme, Wicklow, Ireland), resistant starch (RS) (Berry, 1986) and dietary fiber (Prosky *et al.*, 1992). Moreover, the acidity of the breads was measured as content of H<sup>+</sup> ions by NaOH (0.01 N) titration using phenolphthalein as indicator. A portion from each bread was milled and frozen before analysis. The composition of breads is shown in Table 1.

### 1. *In vitro* study

The bread products were tested *in vitro* to determine the rate of starch hydrolysis following incubation with pepsin and pancreatic  $\alpha$ -amylase (Granfeldt *et al.*, 1992). About 2 g of each sample were dissolved in triplicate in 20 ml of NaCl 0.9% and at 37 °C for 5 h into 500 ml containers filled with 500 ml of 20 mM Na, K-phosphate buffer (pH 6.9) placed in a stirred water bath. Dialysis tubing (13 cm strips) of three different molecular weight cut-offs (3000/6000–8000/12,000–14,000 DA, Spectra Pore no. 132703/132655/132724, width 45 mm, Spectrum Laboratories, Inc., Breda, The Netherlands) has been used. Two aliquots (0.5 ml) from the dialyzed solution were removed for analysis at time 0 and every 30 min. One aliquot was used for the analysis of reducing sugar by the 3,5-

**Table 1**

Percentage composition and titratable acidity of breads (a = wholemeal *S. cerevisiae*; b = wholemeal sourdough; c = white *S. cerevisiae*; d = white sourdough). TS = total starch. RS = resistant starch. The acidity values are expressed as mequivalents of ions H<sup>+</sup>/kg.

	TS	RS (% TS)	Fat	Protein	Fiber	Ash	Moisture	Titratable acidity
a	45	3.3	0.8	8.2	10	2	31	85
b	39	4.7	1.5	7.9	9.8	2.2	37	122
c	51	6.1	5	8.5	2	2	30	53
d	52	7.7	5	7.8	2.2	1.5	30	77

dinitrosalicylic acid method (Miller, 1959) using a maltose standard curve. The second aliquot was used to determine the number of glucose monomers of the permeated fragments. To this purpose, the sample was hydrolyzed using 20  $\mu$ l of 0.5% amyloglucosidase solution (E.C. 3.2.1.3, Sigma MO, USA) at pH 5.6 and the glucose concentration was determined with a glucose analyzer (2300 Stat Plus, YSI Inc., Yellow Springs, USA).

## 2. In vivo study

Eight healthy volunteers (four women and four men), age  $24 \pm 1$  years, BMI  $22.0 \pm 0.5$  kg/m<sup>2</sup> (mean  $\pm$  standard deviation) participated in the study. The bread test meal were provided in amounts corresponding to 50 g of available carbohydrates and served with 500 ml of water (Wolever et al., 1991). The subjects consumed the test products as a breakfast in random order after an overnight fast. Finger prick capillary blood samples were taken before the meal and at 15, 30, 45, 60, 90 and 120 min after the meal. Blood glucose concentration was determined with the YSI glucose analyzer.

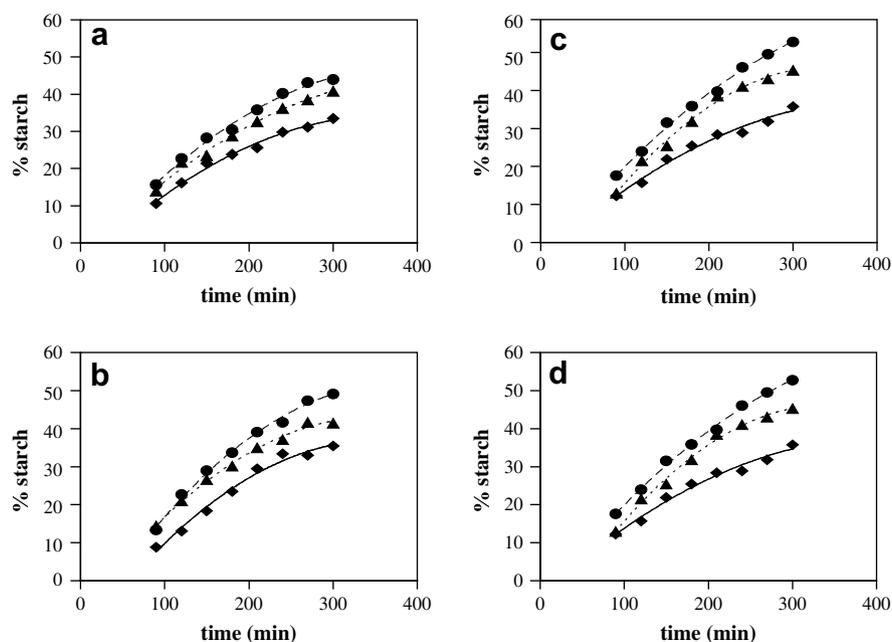
*In vitro* results were submitted to one-way analysis of variance (ANOVA), with Tukey Honest Significant Difference as post-hoc test. *In vivo* results were analysed by two-factors analysis of variance for repeated measures (RM-ANOVA) considering the presence of fiber and the leavening technique as independent factors. Data were expressed as means  $\pm$  SEM setting the level of significance at  $p < 0.05$ .

Sourdough breads showed the highest content of RS compared with breads baked with *S. cerevisiae*, even though the bread

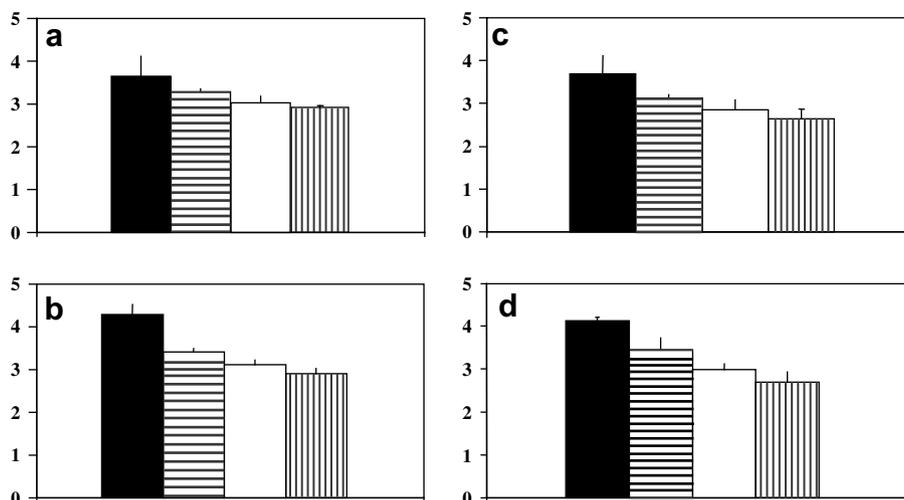
samples were prepared with the same flours. This is in accordance with Brighenti et al. (1998) who observed RS content 20–30% higher in sourdough products when compared to breads traditionally baked with *S. cerevisiae*. Accordingly, Liljeberg et al. (1996) observed an increase of RS content in breads containing increasing concentrations of lactic acid, leading to the hypothesis that the presence of organic acids in bread may increase starch retrogradation and thus RS content. The sourdough breads displayed the highest titratable acidity, compared with the breads baked with *S. cerevisiae*, as well. This is likely attributable to organic acids produced by sourdough microflora during bread making.

Hydrolysis curves expressing the percentage of starch permeated during *in vitro* digestion through the three dialysis tubes of different cut-offs are reported in Fig. 1. The amount of permeated hydrolysis fragments during dialysis is proportional to the membrane cut-off. Nevertheless, no difference could be observed in starch digestion profiles among the four test breads. This is in accordance with Liljeberg and Bjorck (1994) who noted that the hydrolysis rate index obtained with the German pumpernickel sourdough bread was not different from that of current white wheat bread. This observation leads to the conclusion that the leavening technique does not influence starch availability to hydrolytic enzymes in white or wholemeal flour-based breads.

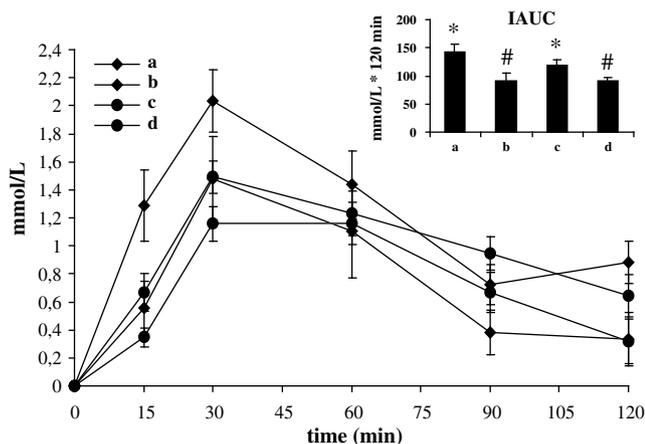
In Fig. 2, the polymerization degree of fragments permeated from dialysis membranes, measured as number of glucose monomers per reducing group, are reported relating to four different time points. No remarkable difference could be appreciated among samples. Apparently,  $\alpha$ -amylase initially digests starch to large polymers that are subsequently reduced to maltose, maltotriose, maltotetraose and dextrans with an average polymerization degree of about 8. As already observed in the literature,  $\alpha$ -amylase breaks starch into 40% maltose, 25% maltotriose, 5% oligosaccharides with 4–8 glucose units and 30% limit dextrans with 8 glucose units (Asp, 1996). Based on these observations, and according to Liljeberg and Bjorck (1994), sourdough does not seem to influence the rate-limit of enzymatic starch digestion, thus suggesting limited debranching activity of the mixed microflora present in the yeast.



**Fig. 1.** Hydrolysis curves, expressing the percentage of starch (digested over total), obtained by three dialysis tubing ( $\blacklozenge$  3000;  $\blacktriangle$  6000–8000;  $\bullet$  12,000–14,000 DA) for each bread (a = whole *S. cerevisiae*; b = whole sourdough; c = white *S. cerevisiae*; d = white sourdough).



**Fig. 2.** Polymerization degree of pieces permeated from dialysis tubing, expressed as number of glucose units *per* equivalent maltose, with cut-off 12,000–14,000 during four different times (■ 90–120 min/▨ 150–180 min/□ 210–240 min/▤ 270–300 min). Data are expressed as mean. Bars represent standard error of the mean. **a** = whole *S. cerevisiae*; **b** = whole sourdough; **c** = white *S. cerevisiae*; **d** = white sourdough.



**Fig. 3.** Incremental blood glucose curves and incremental areas under the curves (IAUC) of postprandial glycemia (**a** = whole *S. cerevisiae*; **b** = whole sourdough; **c** = white *S. cerevisiae*; **d** = white sourdough). Bars with different symbols are significantly different ( $p < 0.001$ ).

Results for the *in vivo* analyses are reported in Fig. 3. The curves represent the incremental blood glucose concentration during the 2 h following the test meal. Histograms represent the total areas under the curves (IAUC) for each meal. Both the response curves and the IAUC values of the sourdough products are lower than the corresponding samples leavened with baking yeast. The statistical analysis shows that the leavening technique significantly affects glucose response when measured as IAUC ( $p < 0.001$ ), whereas fiber content does not ( $p = 0.325$ ).

In conclusion, this study clearly demonstrates that the sourdough leavening technique is able to improve glucose response in healthy subjects. The mechanism remains to be fully characterized, but it is reasonable that organic acids produced by sourdough microflora could delay gastric emptying without influencing starch accessibility or general bioavailability. This, acute effect is similar to that described by direct addition of organic acids to a meal (Brighenti et al., 1995; Liljeberg and Björck, 1998) whereas a mechanism based on fermentation of indigestible carbohydrates

in the colon, as previously described by Brighenti et al. (2006), seems unlikely.

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