# Effect of high-amylose *starch branching enzyme II* wheat mutants on starch digestibility in bread, product quality, postprandial satiety and glycaemic response.

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## Online Supplementary Material

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*Table 1. Certificate of microbiology analysis by ALS Laboratories (UK) Limited - Sands Mill, Huddersfield Road, Mirfield, West Yorkshire, WF14 9DQ*

| Bread type | Method | Test | Result | Units |
|---|---|---|---|---|
| *sbeII* bread | ESGM-M300 | Total Viable Count, 2 days | <10 | cfu / g |
| | ESGM-M303 | Enterobacteriaceae (presumptive) | <10 | cfu / g |
| | ESGM-M304 | Escherichia coli (β-Glucuronidase positive) | <10 | cfu / g |
| | ESGM-M307 | Coagulase positive Staphylococci | <20 | cfu / g |
| | ESGM-M319 | Bacillus cereus (presumptive) | <20 | cfu / g |
| | ESGM-M310 | Clostridium perfringens (presumptive) | <10 | cfu / g |
| | ESGM-M308 | Yeasts | <20 | cfu / g |
| | ESGM-M308 | Moulds | <20 | cfu / g |
| | ESGM-M515 | Salmonella sp. (ELISA) | Not Detected | / 25g |
| WT control bread | ESGM-M300 | Total Viable Count, 2 days | <10 | cfu / g |
| | ESGM-M303 | Enterobacteriaceae (presumptive) | <10 | cfu / g |
| | ESGM-M304 | Escherichia coli (β-Glucuronidase positive) | <10 | cfu / g |
| | ESGM-M307 | Coagulase positive Staphylococci | <20 | cfu / g |
| | ESGM-M319 | Bacillus cereus (presumptive) | <20 | cfu / g |
| | ESGM-M310 | Clostridium perfringens (presumptive) | <10 | cfu / g |
| | ESGM-M308 | Yeasts | <20 | cfu / g |
| | ESGM-M308 | Moulds | <20 | cfu / g |
| | ESGM-M515 | Salmonella sp. (ELISA) | Not Detected | / 25g |

The two genotypes were each sown in eight 6 $m^2$ plots in a randomised block design in in April 2018. Yield and thousand grain weight (TGW) are reported in Table 2 as indication of grain performance when grown in the UK. Kernel weight was determined using a Marvin Seed Analyser (Marvitech GmbH) with a sample of approximately 300 grains per plot. The harvested grains were stored in the field trial station until October 2018, when they were milled into refined flour by Campden BRI using a Bühler mill.

*Table 2. Average kernel weight and yield of sbeIIa/b-AB, sbeIIa-D and WT control wheat. Milling extraction rate is reported here.*

| **Genotype** | **Sowing season** | **TGW (g)** | **Yield (tons/ha)** | **Milling extraction rate (%)** |
|---|---|---|---|---|
| *sbeIIa/b*-AB, *sbeIIa*-D | Spring | 32.2 ± 0.54 | 3.5 ± 0.117 | 73.15 ± 0.30 |
| WT control | Spring | 33.8 ± 0.71 | 3.9 ± 0.16 | 77.97 ± 0.22 |

Means ± SEMs, n = 8.

*Table 3. Bread crust colour analysis*

| Bread type | L* | a* | b* | Hue angle | Chroma |
|---|---|---|---|---|---|
| WT control | 78.2 ± 0.7 | 11.6 ± 0.6 | 40.7 ± 0.8 | 105.7 ± 0.7 | 42.4 ± 0.9 |
| *sbeII* | 83.7 ± 0.9* | 6.7 ± 0.8* | 35.4 ± 1.3 | 100.3 ± 1.1 | 36.1 ± 1.4 |

Mean ± SEMs, n = 4 bread rolls, 16 readings per bread roll (four on each roll side).

Parameter values that are significantly different in the *sbeII* bread compared to the WT control bread are indicated with an asterisk; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (independent groups t-test).

*Table 4. Baseline characteristics measured at screening of the 4 men and 4 women who consumed WT control and sbeII bread*

| Characteristics | Mean | SD | Min | Max |
|---|---|---|---|---|
| Systolic blood pressure (mmHg) | 124 | 10.7 | 108 | 137 |
| Diastolic blood pressure (mmHg) | 81 | 6.1 | 69 | 88 |
| Cholesterol (mmol/L) | 4.8 | 0.9 | 3.1 | 6.2 |
| HDLC (mmol/L) | 1.5 | 0.4 | 0.8 | 2.1 |
| LDLC (mmol/L) | 2.9 | 0.8 | 1.6 | 3.8 |
| Triacylglycerol (mmol/L) | 0.8 | 0.3 | 0.6 | 1.5 |
| Total bilirubin (mmol/L) | 19.4 | 7.6 | 9.0 | 30.0 |
| Total protein (g/L) | 76.0 | 3.3 | 70.0 | 79.0 |
| Albumin (g/L) | 44.0 | 2.9 | 41.0 | 51.0 |
| Globulin (g/L) | 32.0 | 3.4 | 28.0 | 35.0 |
| Alkaline Phosphatase (U/L) | 66.3 | 11.6 | 50.0 | 90.0 |
| Alanine aminotransferase (U/L) | 21.8 | 10.5 | 11.0 | 46.0 |
| Sodium (mmol/L) | 138.1 | 1.1 | 137.0 | 140.0 |
| Potassium (mmol/L) | 4.6 | 0.3 | 4.2 | 5.1 |
| Urea (mmol/L) | 4.3 | 1.2 | 2.6 | 6.7 |
| Creatinine (μmol/L) | 74.5 | 13.7 | 50.0 | 96.0 |

N = 8 of which 4 males and 4 females.

*Table 5. Three days average dietary intake of study participants.*

| **Average of three days dietary intake (per day)** | | | | |
|---|---|---|---|---|
| Energy (kJ) | 9996.8 | 3022.9 | 5819.9 | 15221.1 |
| Energy (kcal) | 2384.7 | 721.3 | 1390.75 | 3631.11 |
| Protein (g) | 94.3 | 27.6 | 56.5 | 138.2 |
| Fat (g) | 104.1 | 29.3 | 69.1 | 163.2 |
| of which saturates (g) | 32.2 | 12.0 | 16.4 | 48.7 |
| Carbohydrates (g) | 250.9 | 87.6 | 118 | 365.5 |
| of which sugars (g | 86.9 | 39.8 | 28.3 | 145.5 |

N = 8 of which 4 males and 4 females. Dietary intake was recorded using the *Libro, 2019* mobile application software. Intake data was analysed using the *Nutritics (2019)* Computer Software, Research Edition (v5.09).

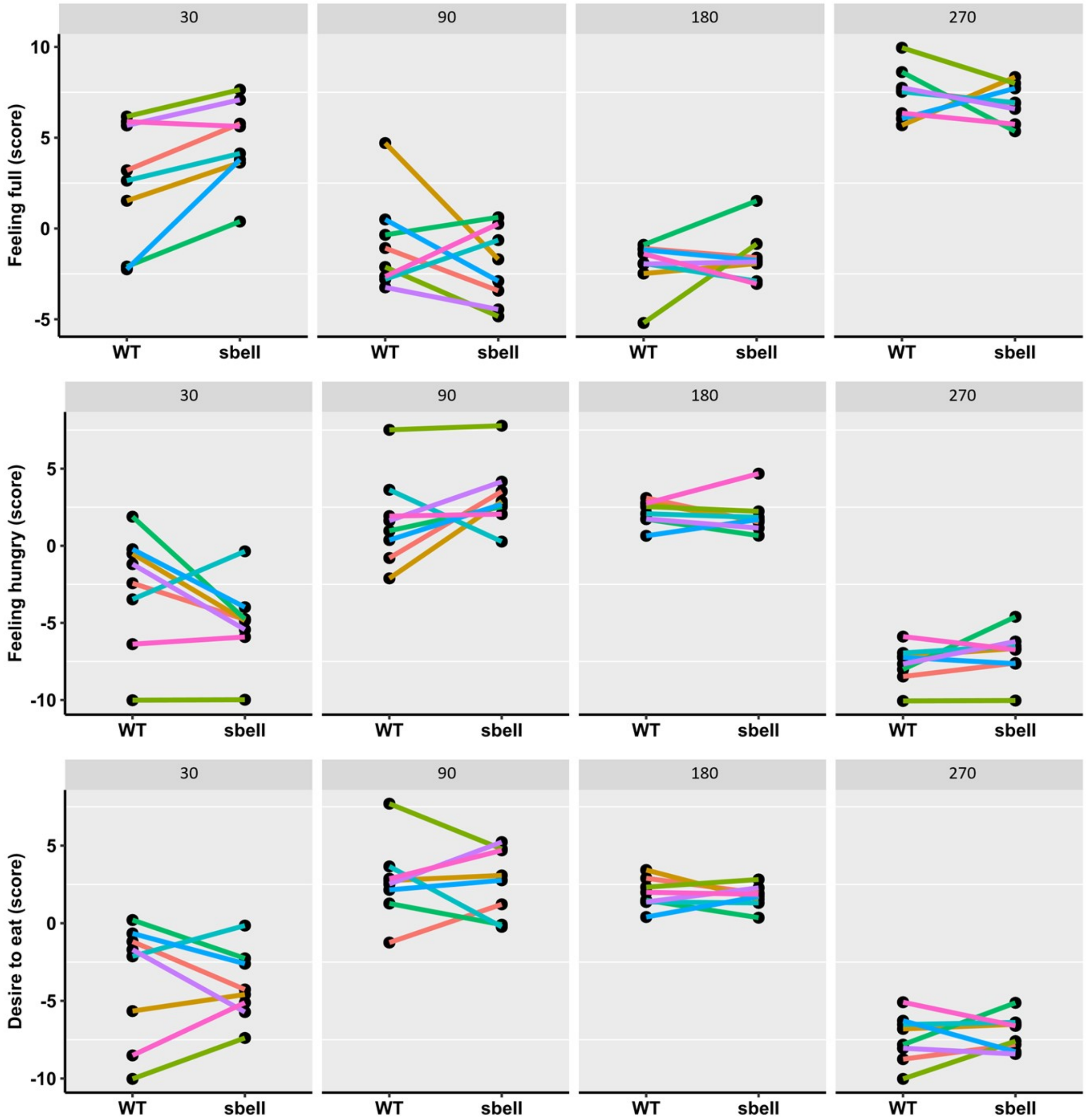


*Figure 1. Individual satiety responses measured by VAS questionnaires for three main indicators reported as the change from baseline, measured at T0 (before consuming the intervention meal): 'Felling hungry', 'Feeling full', 'Desire to eat'. Change from baseline was measured at 30, 90, 180 and 270 minutes after the intervention meal.*

## Preliminary sensory evaluation of *sbeII* bread

Sensory response to *sbeII* bread were compared to the WT control bread using a hedonic scale and 'Just-About-Right' (JAR) scale. The sensory evaluation was not carried out in parallel to accommodate the requirements of the randomized crossover study design.

### *Method and analysis*

Bread palatability was measured after each meal using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely) to determine overall likability, aroma, colour, flavour, moisture, and texture, as well as a JAR scale with five anchor points (1= much too weak to 5 = much too strong).

Two palatability questionnaires were completed after the intervention breakfast, one using a hedonic scale and one using a JAR scale. A 'penalty analysis' was used to determine which attributes of overall palatability (assessment of liking using hedonic scale) were not optimal or JAR, identifying attributes that were most detrimental to the product quality. The overall palatability scores (from the 9-point hedonic scale) were ranked against the JAR scale responses [1]. The penalty score was calculated as $Penalty\ score = \frac{not-JAR}{JAR} \times mean\ decrease\ in\ liking\ (hedonic\ score)$, where 'JAR' is the number of participants that indicated Just-About-Right and 'not-JAR' is the number of participants that indicated either too much or too little for the given attribute on the JAR scale. Any penalty scores greater than 1 indicated that improvement in the product palatability was needed.

***Results and discussion***

The preliminary results on palatability did not reveal major differences in bread liking. The *sbeII* bread scores for overall liking, aroma, flavour, sweetness, texture and size were above neutral on average (score = 5), as the WT control, however the *sbeII* bread appeared to be less moist than the WT control. JAR assessment suggested that participants perceived the *sbeII* bread as *drier* and *doughier* compared to the WT control but overall, it was reasonably 'acceptable' (Supplementary Figure 2).

We explored palatability of *sbeII* bread which, according to the study cohort, was reasonably acceptable compared to the WT wheat control bread roll. The sensory characteristics and consumer acceptability of *sbeII* bread has not yet been rigorously tested and requires further investigation in a non-clinical setting.

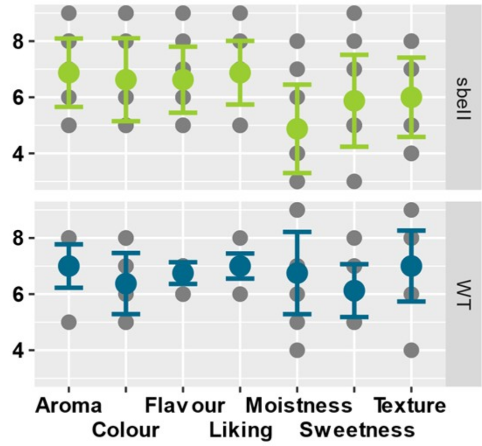


Figure 2. *Palatability scores based on sensory questionnaires, n = 8*

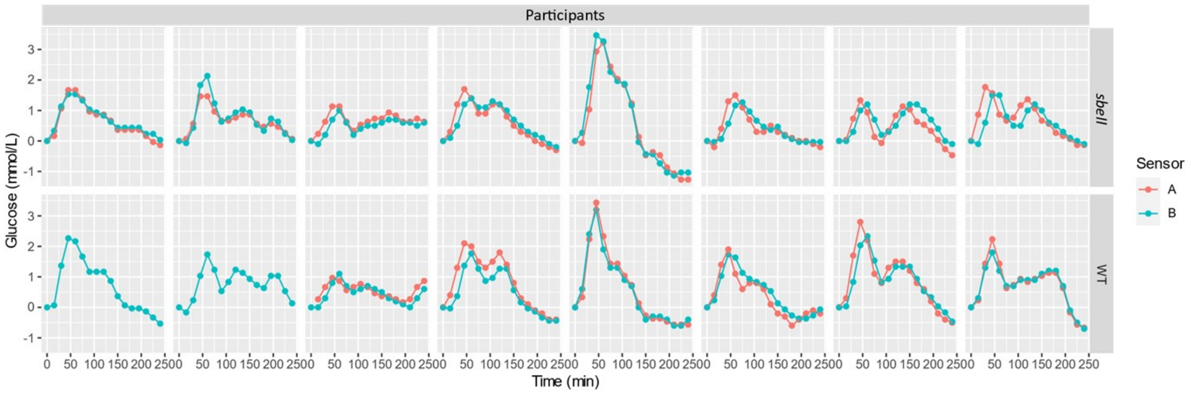


*Figure 3. IF glucose response measured by CGM showing coherent response curve after subtracting the fasting glucose baseline. Two participants lost one of the two sensors and completed the study with one sensor only (bottom left of the figure).*

## References

1. P. Narayanan, B. Chinnasamy, L. Jin and S. Clark, Use of just-about-right scales and penalty analysis to determine appropriate concentrations of stevia sweeteners for vanilla yogurt, *Journal of Dairy Science*, 2014, **97**, 3262-3272.