# Qualitative and Quantitative Oat Betaglucan Assay Methods Based on Congo Red

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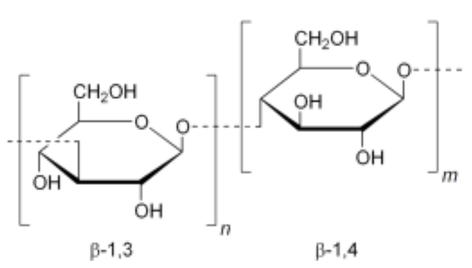
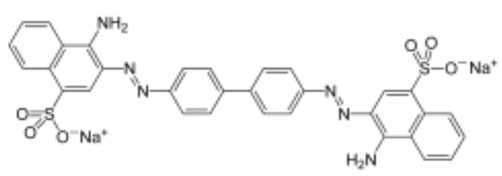
## Background

Oat contains significant amounts of 1-3,1-4 betaglucan (BG), a polysaccharide with proven positive health effects. There is a strong interest to develop oat that produce high levels of BG. Such a development will be facilitated by high throughput analytical methods for screening BG in oat flour. We developed two simple methods for determination of BG content in oat (*Avena sp.*) based on BG and Congo-red (CR) complex formation in solution. This complex has a red color that peaks at 510 nm and can be monitored by a spectrophotometer.



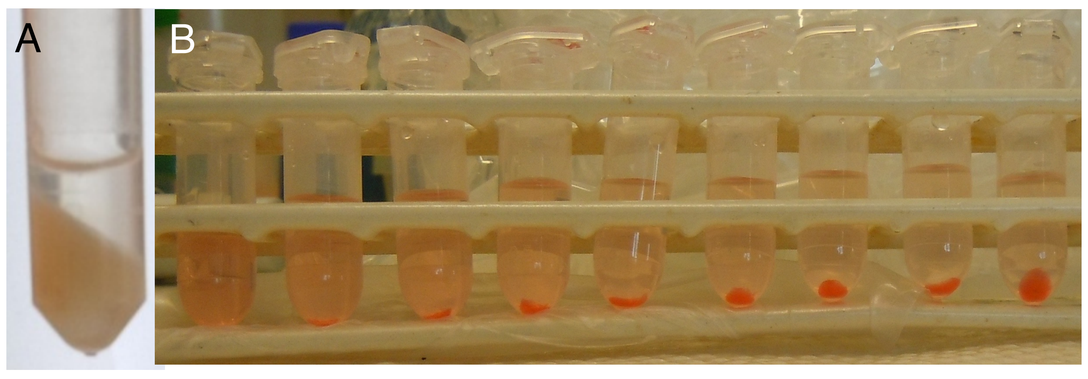

Betaglucan

Congo red




## Qualitative Oat Betaglucan Determination

In the first method the extracted BG solution is mixed with a CR/dextran solution and a precipitate is formed. The original BG concentration can thus be estimated by the size of the red pellet. In addition, since the remaining concentration of CR in the supernatant is inversely correlated the amount of soluble BG that was extracted from the flour, the loss in color can be quantified at 510 nm.




Oat seeds are milled to a fine flour Buffer is added and BG is extracted on a heatblock at 95 °C for 20 min. After cooling and centrifugation a clear supernatant containing BG is obtained (A). In the assay, 0.1 mL sample is taken from the supernatant and mixed with a 1.0 mL CR-dextran reagent, The mixture is centrifuged and a pellet with the BG-CR complex is obtained The figure shows a series of samples with increasingly higher BG levels (B).

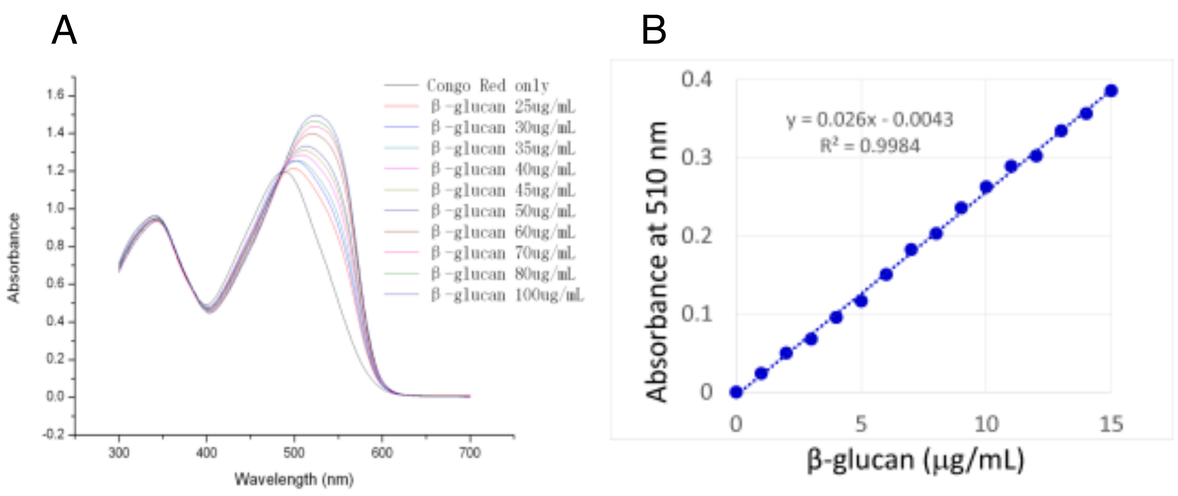
## Beta glucan and Congo Red

It as been known for a long time that betaglucan (BG) and Congo Red (CR) binds and forms a complex in solution, presumably by hydrophobic interactions. Under certain conditions this complex will precipitate (1).

The stability of the complex in combination with a high extinction coefficient can be utilized in various colorimetric assays (2,3). Calcofluor (CAS 4404-43-7) binds also to BG but forms less stable complexes than Congo Red.

## Quantitative Oat Betaglucan Determination

In the second method a limiting concentration of BG is used in the assay. All added BG is then bound to CR and the red color gives a direct measure of the BG concentration. This method has the advantage of high sensitivity and a low cost per assay. It is therefore especially suitable for high-throughput measurement of BG levels in cereal flour.




Total BG was extracted from oat flour with 1M NaOH at room temperature for 30 mins and then neutralized with 1M HCL. After a centrifugation, BG was precipitated from the supernatant with 80% ethanol and the pellet dried and redissolved in buffer. From this mixture, 500 µL was mixed with 500 µL CR working solution. After a few min reaction the BG content was quantified by measuring absorbance at 510 nm. The figure are shown bathochromic effects of increasing BG content (A) and the linearity of the bathochromic shift with the BG concentration (B).

## Conclusions

Screening of betaglucan from oat seeds were performed by two different Congo red methods. One which precipitates the soluble BG and one which measures the total betaglucan. Both methods are easy to perform , have a low cost and are relatively fast (ca 3-4 hours).