# Changes in relative molecular weight distribution of soluble barley beta-glucan during passage through the small intestine of pigs

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**Abstract**

The relative molecular weight distribution of soluble barley beta-glucans (SBB) was monitored through the small intestine in pigs by analyzing water extracts of duodenal- and ileal digesta with HPLC-SEC. Variations among four diets, based on four different barley varieties, were documented as well as variations between animals fed the same diet. The results showed depolymerisation of the SBB throughout the whole small intestine independent of diet. The average molecular weight of the SBB was reduced to approximately 50% in duodenum in all the experimental animals.

**Key words:** beta-glucan; depolymerisation; relative molecular weight distribution; pigs; small intestine

## 1. Introduction

Dietary fiber will affect digestive physiology in pigs and influence digesta flow, voluntary feed intake and thus nutritional absorption and feed digestibility (Bach Knudsen, Hedemann et al. 2012), in addition to manure odor and ammonia emissions (O'Shea, Gahan et al. 2010). Thus, different factors such as grain type and their chemical composition as well as cereal derived endogenous enzyme activities will affect gastrointestinal function, bacteria population and microbial metabolites in the gut (Högberg and Lindberg 2004; Högberg, Lindberg et al. 2004; Bindelle J., Leterme P. et al. 2008; Pieper, Jha et al. 2008). These effects will further depend on the size, solubility and molecular structure of the dietary fiber (Bach Knudsen, Jensen et al. 1993; Glitsø, Brunsgaard et al. 1998; Bach Knudsen, Hedemann et al. 2012).

Dietary fiber, here/often referred to as non-starch polysaccharides (NSP), is depolymerized in the gastrointestinal (GI) tract in different biological systems (Bach Knudsen and Canibe 2000; Coles, Moughan et al. 2005). It is evident that cereal beta-glucans are digested in the upper GI tract of pigs at various degrees, and especially in the distal part of the small intestine (ileum). Digestibility of the cereal beta-glucans will depend on different factors; not only particle size or the feed matrix is important, but also source of beta-glucan and diet composition. Also different grain types and varieties with parallel variation in the fiber content, as well as different biological systems and individual biological differences between subjects will influence the monitored experimental results. However, not only digestibility is important, but physiological properties of beta-glucans are also significant for both animal nutrition and health. Despite different reports on digestion of cereal beta-glucans based on quantitative recovery (Fadel, Newman et al. 1988; Bach Knudsen, Jensen et al. 1993), there is less information on quantitative changes in their molecular weights (Mw). There is a few studies showing changes in the molecular size of oat beta-glucans and of wheat and rye arabinoxylans during digestion in the upper GI tract (Johansen, Wood et al. 1993;

Johansen, Bach Knudsen et al. 1997; Le Gall, Eybye et al. 2010). However, there is scarce information in the literature regarding specific information on the Mw changes of soluble barley beta-glucans during passage in the GI tract and possible variations among/with different barley varieties. This is important since changes in Mw will affect the physico-chemical properties of the beta-glucans significant for their possible influence on gut health in both human and animals.

The main objectives of the present experiment were to measure and document the degree of depolymerization (changes in Mw) of soluble barley beta-glucans in the small intestine of pigs, and study possible differences between different dietary treatments using four barley varieties.

## 2. Material and methods

### *2.1 Dietary treatments*

Four pelleted diets were produced at the Centre for Feed Technology, Ås, Norway. These were based on four Norwegian barley varieties: Olve (normal starch), Marigold (normal starch), Karmosè (high amylose starch) and Magdalena (waxy starch). The barley varieties were grown at the same location (Landvik, Norway) under the same growth conditions in 2010. The diets were formulated to meet the requirements for all nutrients (Subcommittee on Swine Nutrition, Committee on Animal Nutrition et al. 1998). The composition of the diets is given in Table 1.

### *2.2 Experimental animals*

79 The feeding experiment was performed at the Experimental Farm, Department of Animal and
80 Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway. All pigs were
81 cared for according to laws and regulations controlling experiments with live animals in
82 Norway (Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance
83 concerning experiments with animals of January 15, 1996).

84 A total of 16 female pigs ((Norwegian Landrace x Yorkshire) X (Norwegian Landrace
85 x Duroc)) from 4 litters were used in the experiment with an average initial weight at 29.8 kg
86 and an average final weight at 37.6 kg. They were blocked by litter and by live weight, and
87 groups of four animals were fed each experimental diet.

88

89 *2.3 Experimental procedure*

90 The total experimental period lasted for 14 days; a 5-day adaptation period followed by a 9-
91 day experimental period with collection of faeces the last four days. The pigs were given feed
92 twice daily according to a restricted Norwegian feeding scale (Øverland, Granli et al. 2000).
93 The experimental animals were fed in pens designed for individual feeding in a room with an
94 average temperature of 20.4°C, and had free access to water.

95

96 *2.4 Sample collection*

97 The pigs were slaughtered at a commercial slaughter house three hours after the last meal.
98 The digestive tract was separated from the animal at the slaughter line, and the collection of
99 digesta from duodenum and ileum was performed immediately. The duodenal samples were
100 collected from the pyloric ring and 64 cm distally, and the ileal samples from the ileacaecal
101 opening and 64 cm proximally. The samples were put in closed boxes and kept on ice until

being frozen at -20°C. The samples were freeze dried and ground homogenously before being analysed.

### *2.5 Analytical methods*

The four diets were analyzed for yttrium by inductively coupled plasma mass spectrometry (ICP-AES analysis, Perkin-Elmer Optia 3000DV; Perkin-Elmer, Wellesley, MA, USA) at 371 nm, after mineralization and solubilization in acid of the pooled sample.

#### *2.5.1 Extraction of soluble barley beta-glucans for molecular weight determination*

β-Glucans were extracted as described by Rieder et al. (Rieder, Holtekjølen et al. 2012). The initial step involved adding 10 mL of 50% ethanol to a 200 mg sample of the ground diets and of freeze dried duodenal and ileal samples. The mixture was boiled for 15 min., cooled and centrifuged (2000 g, 15 min; Heraeus Multifuge 4 KR). The supernatant was discarded before 20 mL 2.5 mM $CaCl_2$ and 50 μL thermostable α-amylase (Termamyl, Novozymes A/S, Denmark) was added to each sample. The samples were boiled for 90 min. with mixing every 15 min. After cooling, samples were centrifuged (2500 g, 15 min; Heraeus Multifuge 4 KR) and the supernatants collected. Another 10 mL of 2.5 mM $CaCl_2$ was added and the procedure repeated with boiling for 60 min. The supernatants were combined with the previously obtained supernatants and stored frozen before molecular weight analysis.

Content of soluble beta-glucan was calculated as the difference between total beta-glucan and insoluble beta-glucan determined by a mixed-linkage beta-glucan assay kit (Megazyme International Ltd., Wicklow, Ireland). Insoluble beta-glucan was determined in aliquot samples after removal of soluble beta-glucan by extraction.

*2.5.2 Relative estimation of molecular weight distribution of soluble barley beta-glucans (Mw-SBB)*

The apparent molecular weights of soluble barley beta-glucans (hereafter referred to as Mw-SBB) were determined by HPLC-SEC equipped with a post column addition of calcofluor combined with fluorescence detection. The HPLC system consisted of a dual pump system (DIONEX P680) one pump delivering the eluent (50 mM $Na_2SO_4$) at a flow rate of 0.5 mL/min and the other delivering calcofluor (Megazyme International Ltd.) solution (25 mg/L in 0.1 M tris(hydroxymethyl)aminomethane, Sigma, Schnelldorf, Germany) at a flow rate of 0.25 mL/min. A Spectraphysics AS3500 auto injector was coupled to two serially connected columns (Tosho; TSK G6000PWXL + G5000PWXL (7.8mm ID x 30.0cm) in series equipped with a TSK Gel PWXL (6.0mm ID x 4.0cm) guard column).

A T-valve placed in the oven containing the columns (40°C) delivered the calcofluor post column. Injection volume was 20mL and a fluorescent detector (Shimadzu RF-6A, Shimadzu Europa, Duisburg, Germany) was used with 415nm excitation and 445nm emission for detection. The HPLC system was controlled with Chromeleon 6.80 (DIONEX, Sunnyvale, CA, USA).

Beta-glucan Mw standards with average given Mw values s of 35600, 70600, 229000, 26500, 391000 and 650000 were obtained from Megazyme. The standards were solubilised in the eluent (50mM $Na_2SO_4$) added 0.02% $NaN_3$) by boiling for 5 min. and filtered through a Millex-AA filter, syringe-driven filter, 33mm, 0.8µm (Merck Millipore Ltd, Ireland). The standards were then diluted with eluent to give a final concentration of 300µg/mL. A calibration curve based on the Mp (peak molecular weight) of the Mw standards versus their elution volume) was established based on the classical principle of narrow molecular weight

standards. Weight average Mw distributions of the samples were calculated from this using WINGPC-6.2 (PSS) offline using a polynomial fitted standard curve. The classification of the molecular weight distribution into high and low molecular weights (HMw and LMw) was based on dividing the chromatogram in two regions (by elution time); high (20-30 min.) and low (30-42 min.). This cutting point corresponded to ca. 250 kDa in the standard curve.

The calculated weight average Mw's (Mwcalc) only include β-glucan molecules large enough to interact with calcofluor and hence be detected by the resulting fluorescence signal (Rieder, Knutsen et al. 2012). From in-house experiments this cut-off value is approximately 30.000-40.000, but this value is so far not been exactly determined. The reported values therefore do not represent the exact weight average Mw of the samples, but rather the calcofluor based average Mwcalc. Furthermore, since high molecular weight standards (Mw > 650.000) are not available, there is no accurate determination of the molecular weight in the upper range Mw > 650.000). However, for comparative purposes and assessing relative changes in Mw, the methodology was considered appropriate. In fact the unique specificity of the system does not display any or very little interference with starch and other soluble polysaccharides such as arabinoxylan in the system. Cellulose is not soluble and hence not detected.

The SBB were solubilized in water as described by Rieder et al. (Rieder, Holtekjølen et al. 2012) and for the analysis of the actual samples 1.0mL of each water extract was filtered as above and diluted 1:1 with 0.04% $NaN_3$ before injecting into the system. The results of the duodenal and ileal samples are an average of 4 biological replicas. The variation between the technical parallels was less than 10% with a few exceptions.

*2.6 Data analysis*

Analysis of variance and significant differences among means were tested by one-way ANOVA, using Minitab (version 16; Minitab Inc., State College, PA). Significant differences were declared at $P < 0.05$.

## *3. Results and discussions*

### *3.1 Molecular weight distribution of soluble barley beta-glucan (Mw-SBB) in the diets*

The SBB in the four experimental diets exhibited similar monomodal size distribution as seen in Figure 1. The Mw-SBB of the four diets however varied and the diet including the barley variety *Magdalena* (hereafter referred to as Diet-Mag) had a significantly higher average Mw-SBB than the rest of the diets. The diet including the barley variety *Karmosè* (hereafter referred to as Diet-Kar) had the lowest Mw-SBB of the four diets (Fig. 1).

### *3.2 Effect of digestion on molecular weight distribution of soluble barley beta-glucans (Mw-SBB)*

#### *3.2.1 Duodenum – beginning of the small intestine*

The results show a significant depolymerisation of the SBB already at the beginning of the small intestine (duodenum) (Fig. 2). The average molecular weight (average of all diets and all pigs) (AMw-SSB) decreased from approximately 1050 kDa in the diets to ca. 460 kDa in the duodenal samples, a reduction of 55%. There was also a shift in retention time and a broadening of the peak into a bimodal size distribution in the duodenal samples independent of diet (Fig. 2). This showed that the Mw-SBB was depolymerized and that the reduction resulted in two significantly different populations; one population of high molecular weight SBB (HMw-SBB) and one of low molecular weight (LMw-SBB). In the literature there are

196 many studies on fermentation pattern and degradation rate of barley beta-glucans in pigs.
197 However, there is scarce information regarding changes in molecular weight of barley beta-
198 glucans. For oat beta-glucans similar depolymerisation pattern has been observed (Johansen,
199 Wood et al. 1993; Johansen, Bach Knudsen et al. 1997).

200 The average HMw-SBB size distribution (as average of all diets and all pigs) was 940
201 kDa and it accounted for ca. 45% of the molecular size distribution in the duodenal samples,
202 while the average LMw-SBB was 105 kDa with a 55% share. Also oat beta-glucans showed
203 depolymerisation in the upper small intestine of pigs (up to 55%) (Johansen, Bach Knudsen et
204 al. 1997).

205 All diets showed the same change into a bimodal size distribution. Still, some
206 significant differences were seen depending on the diet. Overall, diet-Mag had the
207 significantly highest average Mw-SBB, followed by diet-Kar and diet-Olv, with diet-Mar
208 having the lowest. Also, the portion of high molecular weight SBB (HMw-SBB) differed and
209 the largest part of HMw-SBB was found in the Diet-Mag (51%), while diet-Mar had the
210 lowest (32%).

211

212 *3.2.2 Ileum – end of the small intestine*

213 The average molecular weight of SBB in ileum showed a significant decrease
214 compared with the duodenal samples, from 460 kDa to 250 kDa respectively ($P < 0.05$). The
215 corresponding decrease in AMw-SBB compared to the original diets was 75%.

216 The results showed that the SBB was depolymerized throughout the small intestine
217 with a shift towards a higher portion of LMw-SBB in the ileal samples (Fig. 3) compared with
218 the duodenal samples. Thus, the low molecular weight portion increased moving through the
219 small intestine from the duodenum to the ileum. The share of HMw-SBB decreased equally,
220 and again, diet-Mag had the highest Mw-SBB and the largest portion of HMw-SBB (only

28%) in the ileal samples, with diet-Mar the lowest (15%) (Fig. 3). This is consistent with findings for oat. Johansen et al. (1997) showed an increased depolymerisation for oat beta-glucans going from the proximal to the distal small intestine in pigs. Thus, the oat beta-glucans in the distal small intestine after 3h post-prandial showed higher depolymerisation, decreasing the share of high Mw oat beta-glucan.

### *3.3 Variations among pigs in distribution of molecular weight distribution of soluble barley beta-glucans (Mw-SBB) in the duodenal and ileal samples*

Some variations were seen among the experimental animals fed the same diet (see figure 4 and 5). Figure 4 shows the variations found in the duodenal samples within pigs fed Diet-Mag, while Figure 5 shows the variation among the ileal samples of the pigs fed the Diet-Mag. The observed variations among pigs fed the same diet might relate to differences in the microorganisms present in their gastrointestinal tract. It could also be associated with variation in the matrix of the pellets after chewing as well as different drinking pattern. The variations between the biological parallels make it important to include a sufficient number of biological parallels to obtain reliable data as well as to verify the results when working with animals and animal trials. Still, despite some variation among pigs fed the same diet, the effect on SBB is evident. The molecular weight of the SBB is reduced and the depolymerisation starts at duodenum and continues all the way through the small intestine. At ileum the Mw-SBB is reduced up to 80% compared to the original diet.

## ***4. Conclusion***

Soluble barley beta-glucan (SBB) is depolymerized during digestion in pigs and there is a significant depolymerisation of SBB naturally occurring already in the upper GI tract, in the

245 small intestine. Our results show that depending on variety, the SBB is depolymerized up to
246 60% in the duodenum and 80% in the ileum. Thus, before the SBB has reached the hindgut
247 and is fermented, its Mw has already been significantly reduced into a larger share of low
248 molecular weight SBB (ca. 100 kDa). The depolymerisation of the beta-glucan might be due
249 to hydrolytic enzymes excreted by microbiota in the upper digestive tract of the
250 animal. However, retained endogenous hydrolase activities in the barley material may be
251 present despite barley processing and transit through the upper GI-tract.

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## Conflict of interest statement

The authors confirm no conflict of interest with this article.

## Tables

### Table 1. Composition of the four diets and their amount of soluble beta-glucan (%)

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| Barley *Marigold* | 83.47 | | | |
| Barley *Magdalena* | | 83.47 | | |
| Barley *Karmosè* | | | 83.47 | |
| Barley *Olve* | | | | 83.47 |
| Soybean meal (HiPro) | 15.0 | 15.0 | 15.0 | 15.0 |
| Limestone meal ($CaCO_3$) | 1.3 | 1.3 | 1.3 | 1.3 |
| Mineral premix | 0.16 | 0.16 | 0.16 | 0.16 |
| Vitamin premix | 0.06 | 0.06 | 0.06 | 0.06 |
| $Y_2O_3$* | 0.01 | 0.01 | 0.01 | 0.01 |
| Soluble beta-glucan | 1.6 | 3.0 | 2.6 | 2.6 |

*Yttrium oxide was used as the indigestible dietary marker.

**Figure legends**

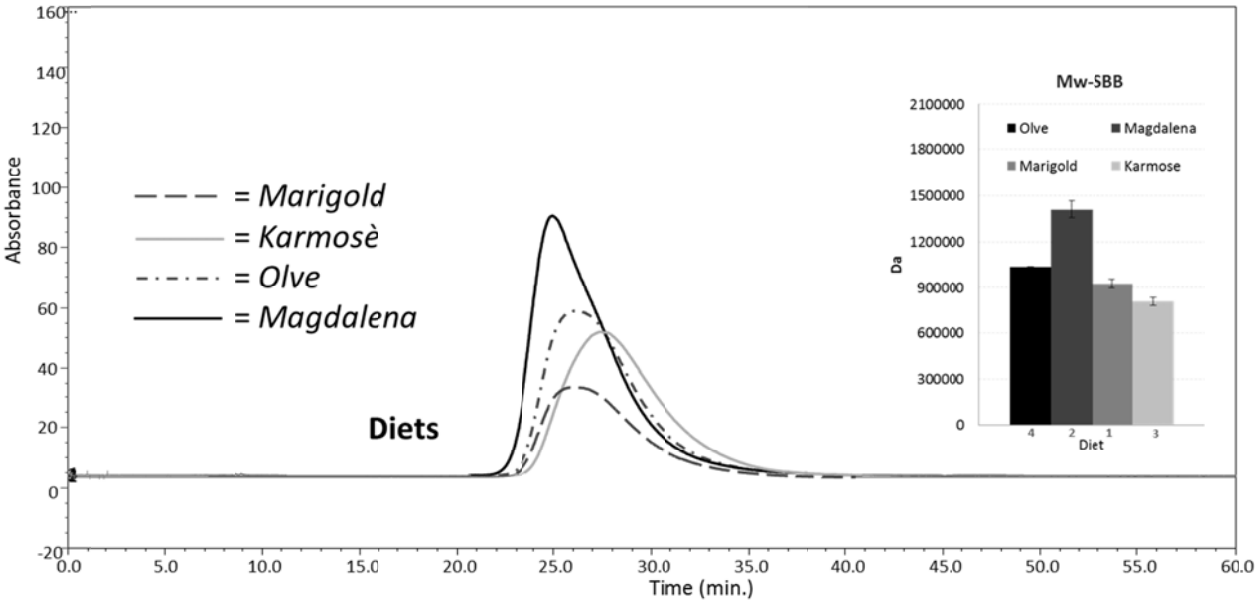
**Figure 1:** Chromatogram showing the relative molecular weight profile of the soluble barley beta-glucans (Mw-SBB) in the diets based on the different barley varieties including their calculated average Mw-SBB as bar graphs. The error bars represent the standard deviations (two technical parallels).

**Figure 2:** Chromatogram showing the relative molecular weight profile of the soluble barley beta-glucans (Mw-SBB) in the different duodenal samples including their calculated average Mw-SBB as bar graphs. The degree of depolymerisation compared to the Mw-SBB in the corresponding diets are given (in %) above the bars. The error bars represent the standard deviations (four biological replicas (pigs)).

**Figure 3:** Chromatogram showing the relative molecular weight profile of the soluble barley beta-glucans (Mw-SBB) in the different ileal samples including their calculated average Mw-SBB as bar graphs. The degree of depolymerisation compared to the Mw-SBB in the corresponding diets are given (in %) above the bars. The error bars represent the standard deviations (four biological replicas (pigs)).

**Figure 4:** Example of the variation found in the relative molecular weight profile of duodenal samples among the four pigs (1-4) fed the same diet (Magdalena). The two overlapping chromatograms represent the two technical parallels.

**Figure 5:** Example of the variation found in the relative molecular weight profile of ileal samples among the four pigs (1-4) fed the same diet (Magdalena). The two overlapping chromatograms represent the two technical parallels.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Time (min.)
= Marigold
= Karmosè
= Olve
= Magdalena
Diets
Mw-SBB
Olve
Magdalena
Marigold
Karmose
Da
Diet

**Figure 1**

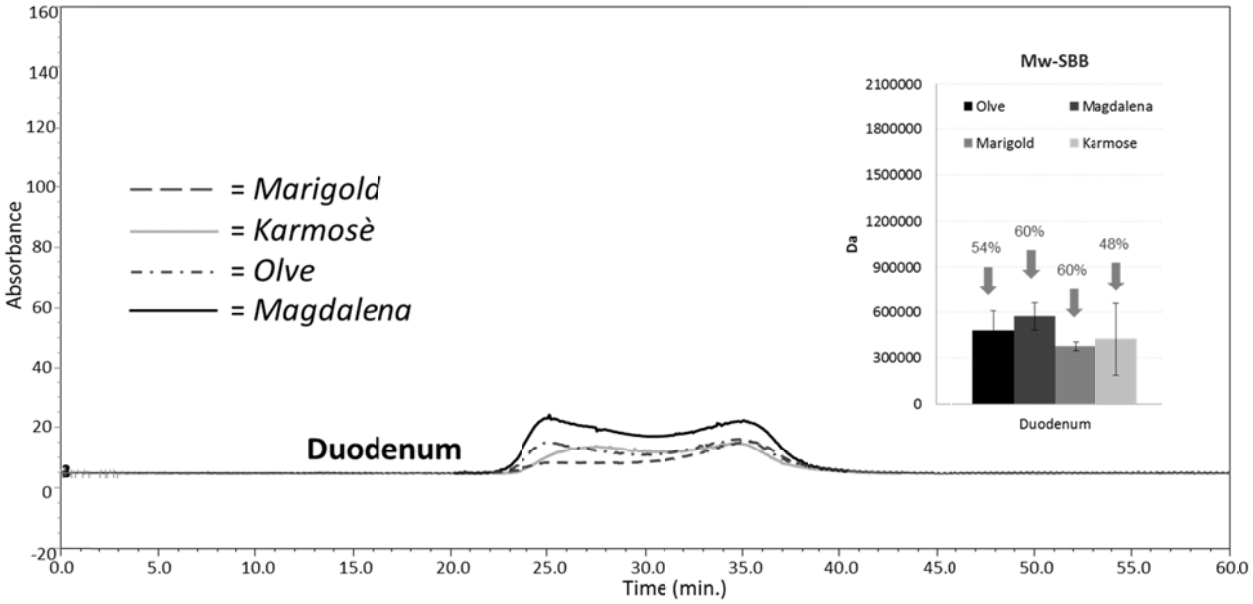
Absorbance
Time (min.)
= Marigold
= Karmosè
= Olve
= Magdalena
Duodenum
Mw-SBB
Olve
Magdalena
Marigold
Karmose
Da
54%
60%
60%
48%
Duodenum

**Figure 2**

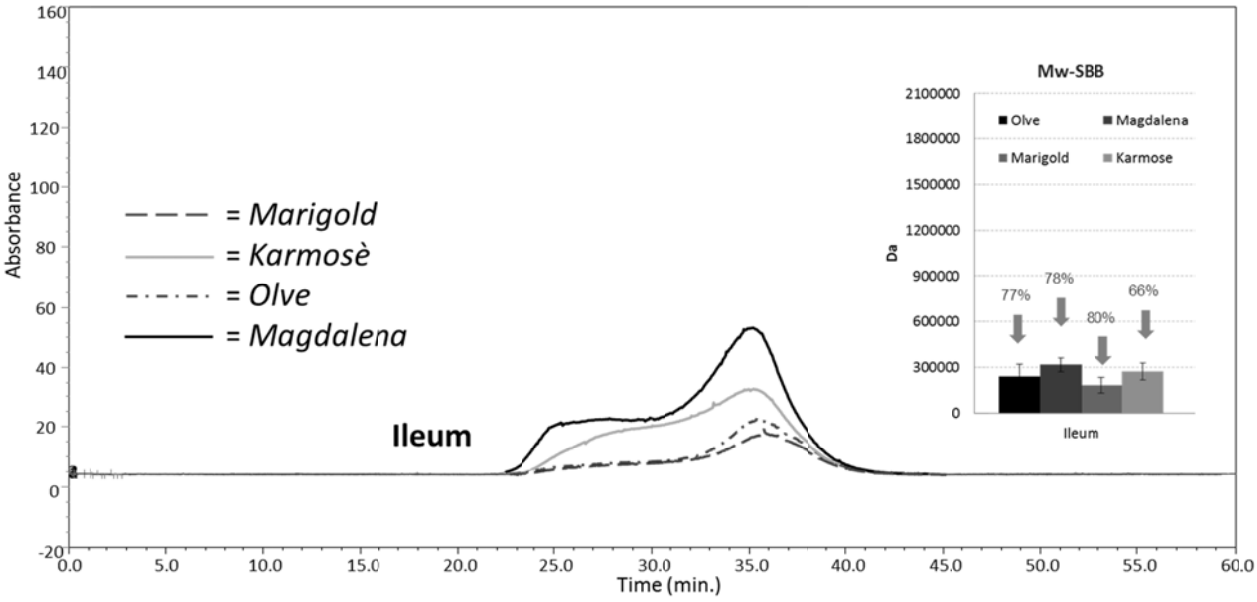
Absorbance
Time (min.)
= Marigold
= Karmosè
= Olve
= Magdalena
Ileum
Mw-SBB
Olve
Magdalena
Marigold
Karmose
Da
77%
78%
80%
66%
Ileum

**Figure 3**

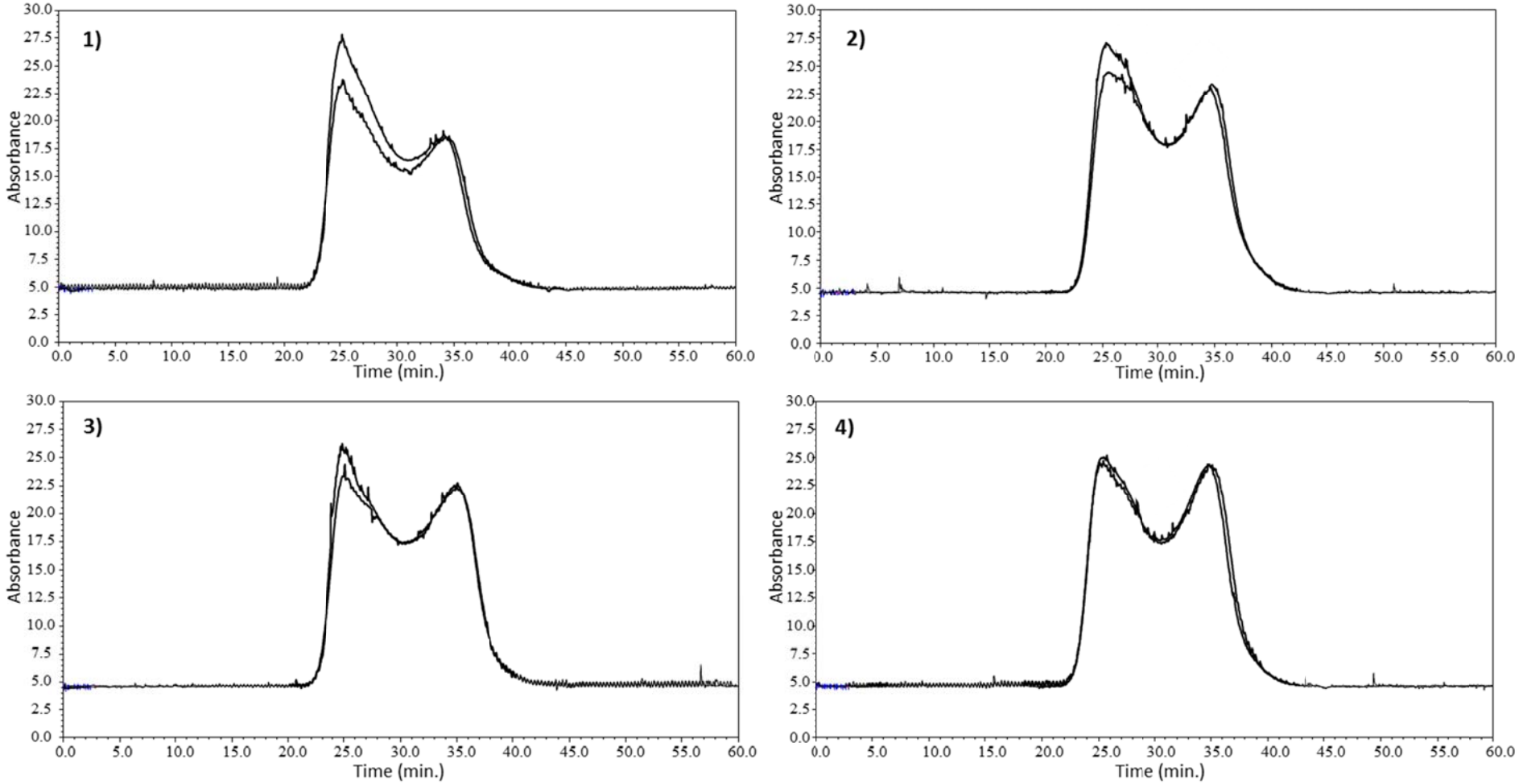


**Figure 4**

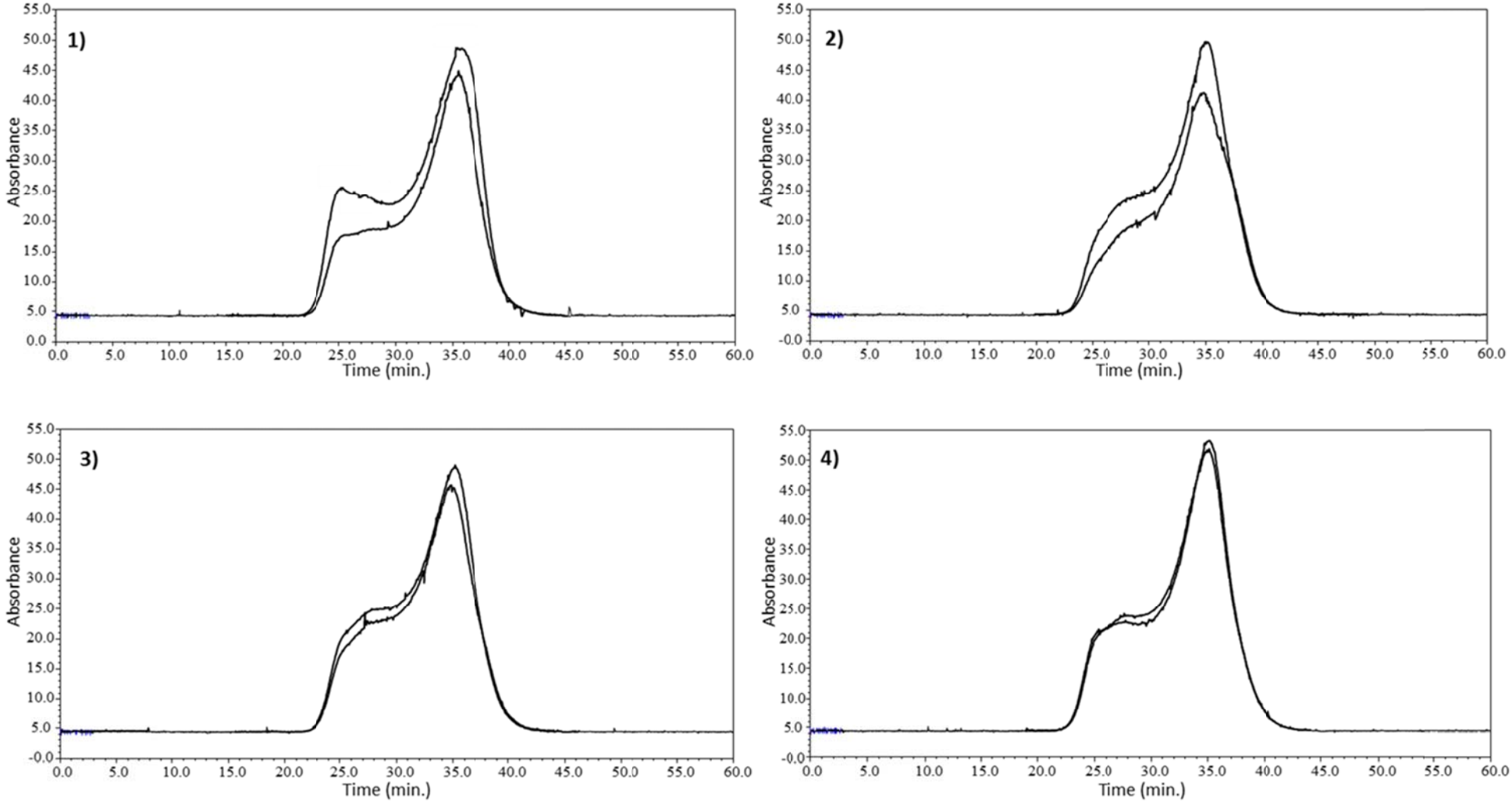


**Figure 5**

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