Structural Features and Complement-Fixing Activity of Pectin from Three *Brassica oleracea* Varieties: White Cabbage, Kale, and Red Kale

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Leaves of different cabbage species are used both as food and as wound healing remedies in traditional medicine. This supposed wound healing activity might be connected to presence of immunomodulating water soluble polysaccharides. To study this, three different cabbage varieties, white cabbage (W), kale (K), and red kale (RK), were pretreated with 80% ethanol and then extracted with water at 50 °C and 100 °C for isolation of polysaccharide-containing fractions. The fractions were analyzed for monosaccharide composition, glycosidic linkages, M_w distribution, protein content, and phenolic compounds and then tested for complement-fixing activity. All fractions contained pectin type polysaccharides with linkages corresponding to homogalacturonan and hairy regions. Those extracted at 50 °C contained higher amounts of neutral side chains and were more active in the complement-fixation test than those extracted at 100 °C. The fractions can be ranged by decreasing activity: K-50 > RK-50 > W-50 ≈ K-100 > RK100 ≈ W-100. Studies on structure−activity relationships (SAR) employing multivariate statistical analysis strongly suggest that the magnitude of the measured activity is influenced by the content of certain side chains in the polymers. High activity correlates to large neutral side chains with high amounts of (1→6)- and (1→3,6)-linked Gal and low amounts of (1→4)-linked GalA but not on molecular weight distribution of the polymers.

Introduction

Cabbage is a vegetable that is widely used in households. Traditionally in Norway, white cabbage (*Brassica oleracea* var. *Capitata*) has been the most commonly used species, but during recent years, other cabbage varieties such as kale (*Brassica oleracea* var. *Sabellica*) have been introduced with increasing popularity. Cabbage leaves are also used in traditional medicine. They are used externally both as a wound healing remedy[1] and for pain relief in joints, especially in the knee.[1−3] In previous work on *Plantago major* leaves, which is another plant used for wound healing, we found pectin type polysaccharides with immunostimulating properties.[4−7] It is suggested that such polysaccharides may enhance the wound healing process through stimulation of the innate immune system.

Pectin type polysaccharides seem to be constituents of the cell walls of all dicotyledonous plants[8] including white cabbage.[9] Pectin consists of unbranched homogalacturonan regions and branched so-called hairy regions.[10] There are indications that the hairy regions of several pectins may express immunostimulating activities,[6,11,12] but the general nature of this is not yet proven. In the present study the complement-fixing potential of the polysaccharides from three cabbage varieties; white cabbage, kale, and a red cultivar of kale will add knowledge to this phenomenon. In order to elucidate the possible structure−activity relationships related to complement-fixing activity, multivariate statistics is used as a rational approach. This tool reaches further than a semiquantitative approach only based on linearity between activity and one structural element at a time. To our knowledge this is the first time studies of structure−activity relationships on the complement-fixing activity have been supported by multivariate statistical analysis.

Materials and Methods

Plant Material. White cabbage (*Brassica oleracea* var. *Capitata* cultivar Bartolo), kale (*B. oleracea* var. *Sabellica* cultivar green Moskruset), and a red cultivar of kale (*B. oleracea* var. *Sabellica* cultivar Redbor) were cultivated in Ås, Akershus at Vollebekk testfield. Fresh kale leaves were crushed in liquid nitrogen and stored in 96% ethanol at −20 °C until extraction.

Extraction. Fresh white cabbage leaves were cut into 3−5 mm slices and extracted with 80% ethanol at 80 °C under reflux and continuous stirring for 2 h and then filtered through gauze. Crushed leaves from kale and red kale were extracted in the same manner. The extraction was repeated twice, i.e., until the plant material became colorless. The insoluble material was dried at ambient temperature and extracted with distilled water at 50 °C under reflux and stirring for 3 h. After filtration through four layers of gauze and then through a Whatman GF/A glass fiber filter, the extracts were subjected to dialysis (M_w cut off 3500 Da) against distilled water. The dialysis water was changed several times until negative reaction of the phenol−sulfuric acid test of Dubois.[13] Finally the extracts were lyophilized. The cabbage residues were subsequently extracted with distilled water at 100 °C under reflux and stirring, and the soluble fractions were filtered, dialyzed, and lyophilized as described above. Fractions from white cabbage extracted

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at 50 °C and 100 °C were designated W-50 and W-100, respectively, the two from red kale RK-50 and RK-100, and the two from kale K-50 and K-100.

Monosaccharide Composition. Methanolysis was performed according to the method of Chambers and Clamp[14] with modifications as previously described[5] using 4 M HCl in anhydrous methanol for 24h at 80 °C.

Linkage Analysis. Prior to methylation, uronic acids present in polymers were reduced with sodium borodeuteride.[15] The polysaccharides were then subjected to methylation using NaOH in DMSO for sugar alkoxide formation.[16] After methylation, hydrolysis, reduction, and acetylation, the partially methylated alditol acetates were analyzed by GC-MS using a Fisons GC 8065 with a SPB-1 fused silica capillary column (3 m × 0.2 mm i.d.) with 0.2 μm film thickness. EI mass spectra were obtained with a Hewlett-Packard Mass Selective Detector 5970. The injector temperature was 250 °C, and the detector temperature was 300 °C. The column temperature was 80 °C when injected and then increased with 30 °C/min until 170 °C, followed by 0.5 °C/min to 200 °C and then 30 °C/min to 300 °C. Helium was the carrier gas. The quantification was based on integration of peaks in the GC-MS spectra and relating these to the total amount of each monosaccharide found by methanolysis.

Protein Content. The protein content was determined by the method of Lowry[17] modified by Peterson[18] using bovine serum albumin as standard.

Phenolic Compounds. Detection of phenolic compounds was performed with the Folin Ciocalteus reagent according to the method described by Swain and Hillis[19] using ferulic acid as standard.

^{13}C NMR. Samples (15 mg) were dissolved in 50 mM ammonium oxalate by incubating in a water bath at 60 °C for 5 min. After centrifugation at 16 000*g* for 5 min, 1 mL of the supernatant was transferred to a NMR tube (Norell no 506-P precision). ^{13}C NMR spectra were recorded on a Varian 300 MHz instrument at 80 °C, 2 s pulse delay, 1.64 s acquisition time and at 86° pulse angle, sweep width 15974 Hz, and approximately 60000 data points.

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was performed with three Agilent Aquagel-OH (40, 50, and 60) columns coupled in series. The columns were connected to a Shimadzu system with System controller (SCL10A), degassing unit (DGU3A), auto injector (SIL 10A), liquid chromatograph (LC10AD), column oven (CTO 10A), refractive index detector (RID6A). A 50 mM Na_2SO_4 solution was used as eluent with 0.8 mL/min flow rate. Pullulans from Polymer Laboratories LTD were used as standards (5.8, 12.2, 23.7, 48, 100, 186, 380, 853, and 1600 kDa). Molecular weights of *Brassica* polysaccharides were calculated with PSS WinGPC program.

Complement-Fixing Test. As a measure of influence on the human immune system, the fractions were analyzed for activity in the complement-fixation test according to the method of Michaelsen et al.[7] Briefly, human serum as complement source was incubated with the cabbage fractions, and their influence on the human complement system was measured in a hemolysis inhibition system. The fractions can either activate complement or inhibit complement factors. In both situations complement activity is depleted with a negative influence on a balanced hemolysis system involving antibody-sensitized sheep red blood cells and a human serum diluted to give 50% hemolysis. The activity is recorded as ICH_{50} which is the concentration of the fraction needed to inhibit 50% of the lysis. The lower the ICH_{50} value, the higher the complement-fixing activity. The degree of hemolysis was measured as absorbency at 405 nm. The extracts were tested in quadruplicates. PMII, a polysaccharide fraction from *Plantago major* L.[5,6] was used as positive control.

Statistical Analysis. Both the correlation analyses between activity and structure and the one-way analysis of variance (ANOVA), for testing significant differences between the activities of the extracts, were performed with Minitab (version 14.2; Minitab Inc, State College, PA). *P*-values of 0.05 or less were considered significant. The relationships between the chemical parameters (*x*-variables) and the ICH_{50} value (*y*-variable) were studied by partial least-squares regression (PLSR) using the Unscrambler software package (Version 9.2; CAMO A/S, Trondheim, Norway). A cross-validation combined with a modified Jack-knifing (uncertainty test) was used to identify significant *x*-variables for the prediction of the *y*-variable.

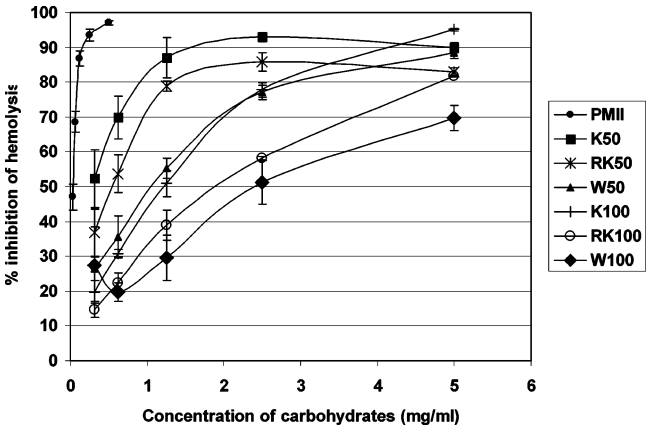

Figure 1. Complement-fixing activity of *Brassica* fractions. PMII = positive control derived from *Plantago major* L. The concentrations are adjusted to the total carbohydrate content of the samples.

Results and Discussion

Complement-Fixing Activity. All extracts tested had some activity in the complement-fixation test (Figure 1), but were less active than the positive control PMII. K-50 was the most active *Brassica* fraction showing a calculated 50% inhibition of hemolysis (ICH_{50} value) at 290 μg/mL (Table 1). K-50 was significantly more active than RK-50 ($p = 0.004$), and RK-50 showed higher activity than W-50 ($p = 0.001$). Neither the ICH_{50} values of W-50 and K-100 nor those of RK-100 and W-100 showed significant differences. The latter two had lower activity than K-100 ($p = 0.005$ and $p = 0.012$, respectively). On the basis of ICH_{50} values, the fractions can be ranged as follows with decreasing activity: K-50 > RK-50 > W-50 ≈ K-100 > RK100 ≈ W-100. Possible contamination with bacterial lipopolysaccharide (LPS) does not interfere with the test system. This has been shown previously with LPS from *E. coli* and *N. miningitidis* at a concentration range 3−750 μg/mL.[20]

Fraction Composition. All freeze-dried fractions showed the same gross contents: 40−60% carbohydrate, 1−2% protein, and less than 1% phenolic compounds (Table 1). According to the results from methanolysis, the polysaccharides present contain arabinose, rhamnose, galactose, and galacturonic acid which are typical components of pectin polysaccharides.[10] In addition, minor amounts of mannose, xylose, fucose, glucuronic acid, and glucose were detected. These latter monomers may come from hemicelluloses present.

K-50, RK-50, and W-50 contain material with higher M_w compared to the corresponding fractions isolated at 100 °C (Table 1). A certain degree of depolymerization due to β-elimination may have occurred since extraction was performed without pH control, at elevated temperatures, and in addition, ^{13}C NMR showed that the pectin present had a relatively high degree of methyl esterification, all favoring β-elimination.[21,22] In our experience, when extraction is performed at pH 4.5 at room temperature for avoiding β-elimination, arabinofuranosidic linkages are hydrolyzed (unpublished results). The arabinose residues are significant components of the neutral side chains in pectic polysaccharides of the rhamnogalacturonan-I type.[10]

Table 1. Monosaccharide Composition (w/w % of total carbohydrate content), Contents of Proteins and Phenolic Compounds (w/w %), Molecular Weight Distribution (M_w, M_n), Polydispersity (M_w/M_n), ICH_{50} Value in the Complement-Fixation Test

	K-50	RK-50	W-50	K-100	RK-100	W-100
Ara	23.6	20.0	15.6	10.7	11.7	16.9
Rha	3.3	4.4	3.6	3.8	4.7	5.0
Fuc	1.9	2.3	0.5	0.9	0.8	0.6
Xyl	2.3	1.7	2.5	2.1	1.3	3.3
Man	3.9	2.7	1.9	1.8	0.4	3.5
Gal	23.1	18.1	12.8	9.0	8.5	8.4
Glc	3.6	3.3	4.4	3.1	2.5	5.0
GalA	38.5	47.4	58.5	68.6	69.6	57.1
GlcA	0.1	0.1	0.1	0.1	0.1	0.1
protein	1.7	1.5	1.3	2.1	1.9	1.9
phenolic compounds	0.8	0.9	0.6	0.1	0.3	0.0
M_w (kDa)	133	127	125	70	29	70
M_n (kDa)	17	15	65	30	11	30
M_w/M_n	7.8	8.5	1.9	2.3	2.6	2.3
ICH_{50} value (mg/ mL)[a]	0.29 ± 0.08	0.53 ± 0.10	1.04 ± 0.04	1.20 ± 0.14	1.82 ± 0.12	2.45 ± 0.47

[a] Mean ($n = 4$) ± SD.

Table 2. Glycosidic Linkages Present (% of total carbohydrate content)

	K-50	RK-50	W-50	K-100	RK-100	W-100
T-Ara*f*	10.2	7.2	6.0	4.4	4.2	8.3
1,2-Ara*f*	1.6	3.3	0.8	0.5	0.2	0.2
1,3-Ara*f*	1.6	0.0	0.4	0.1	0.2	0.2
1,5-Ara*f*	6.4	6.7	7.1	4.1	5.4	6.5
1,2,5-Ara*f*	3.8	2.8	1.4	1.7	1.6	1.8
T-Xyl*p*	1.5	1.0	1.0	1.5	0.8	1.7
1,4-Xyl*p*	0.3	0.1	0.8	0.0	0.1	0.5
1,3,4-Xyl*p*	0.5	0.5	0.7	0.6	0.4	1.1
T-Rha*p*	0.9	0.8	0.8	1.3	1.2	0.9
1,2-Rha*p*	1.6	2.6	1.8	1.5	2.1	2.4
1,3-Rha*p*	0.2	0.2	0.1	0.2	0.2	0.1
1,2,4-Rha*p*	0.7	0.8	0.9	0.9	1.2	1.6
T-Gal*p*	3.6	4.1	4.2	3.3	2.9	4.9
1,3-Gal*p*	2.2	1.6	2.0	0.9	1.3	1.0
1,6-Gal*p*	4.0	2.8	1.5	1.0	1.0	0.7
1,3,6-Gal*p*	13.3	9.5	5.1	3.7	3.3	1.8
T-Gal*p*A	1.2	1.5	2.7	3.2	3.1	1.8
1,4-Gal*p*A	36.5	44.8	54.7	63.5	64.4	53.1
1,3,4-Gal*p*A	0.5	0.6	0.7	1.4	1.3	1.5
1,2,4-Gal*p*A	0.3	0.5	0.4	0.5	0.8	0.6
T-Fuc*p*	1.7	2.1	0.5	0.9	0.8	0.6
1,3 Fuc*p*	0.2	0.2	0.0	0.0	0.0	0.0
T-Glc*p*A	0.1	0.1	0.1	0.1	0.1	0.1
T-Glc*p*	1.0	0.4	0.8	0.2	0.3	0.2
1,4-Glc*p*	2.2	2.9	3.6	2.9	2.2	4.1
1,4,6-Glc*p*	0.5	0.0	0.0	0.0	0.0	0.6

Hydrolysis of arabinose residues is unwanted because the neutral side chains are considered as the active parts of the pectic molecules expressing immunostimulatory activity while the homogalacturonan regions that are degraded during β-elimination do not express such activity.[6,11] This may explain why bioactive pectic polysaccharides are successfully extracted with water without pH control.

Polysaccharide Structure. Linkage analysis (Table 2) revealed the presence of linkages typical for pectin type polysaccharides that are composed of homogalacturonan regions and hairy regions (Rhamnogalacturonan I).[10] All fractions contain similar types of pectin structures and are composed of high amounts of (1→4)-linked Gal*p*A residues with minor amounts of branching in position 2 and 3. Rhamnose residues are (1→2)- and (1→2,4)-linked and are considered as part of the backbone in pectin polysaccharides with neutral side chains composed of arabinogalactans, galactans, and arabinans attached to O-4 of Rha*p*. Methylation analysis suggested that the neutral side chains are composed of (1→3)-, (1→6)-, and (1→3,6)-linked Gal*p* residues and that most of the Ara*f* residues are (1→5)-linked, terminally linked, and (1→2,5)-linked.

Supposing that all neutral side chains are linked to *O*-4 of (1→2)-linked Rha*p* residues, we can estimate the average size of the side chains (SC) by considering the ratio [(Gal + Ara)/(1→2,4)-Rha*p*] of the different fractions. Following this, the fractions extracted at 50 °C seem to contain larger side chains compared to those extracted at 100 °C. Minor amounts of terminally linked Xyl, Fuc, and GlcA were also detected in addition to some (1→4)-linked Glc.

According to the ^{13}C NMR spectra (Figure 2), all fractions contain more or less the same components as found by methylation analysis. Anomer signals from α-Ara appear in the region 108.3−109.9 ppm; C-1 from terminally linked α-Ara appear at 109.9, and C-1 from (1→5)-linked α-Ara is found at 108.4 ppm.[23] Signals at 88.1 ppm are from C-2 and those at 67.8 ppm from C-5 of (1→2)- and (1→2,5)-linked α-Ara, respectively.[23] The peaks at 84.9 and 62.1 ppm are from α-Ara C-4 and C-5 of terminally linked α-Ara, respectively. The anomer signals at 104 ppm are from C-1 of β-Gal.[24] C-3 signals from (1→3)-linked β-Gal appear at 83 ppm, and C-3 signals from (1→6)-linked β-Gal are found at about 73.6 ppm. C-6 from (1→6)- and (1→3,6)-linked β-Gal give rise to signals at 70.7 ppm while the signal for C-6 from (1→3)-linked β-Gal superimposes the C-5 α-Ara signals at 62.1 ppm.[24]

At 100.9 and 100.1 ppm, large anomer signals from esterified and nonesterified α-GalA residues appear. Due to the relative sizes of these signals, all fractions contain highly esterified pectin, also shown by the large *O*-Me signal at 53.6 ppm. The C-4 signals from α-GalA are found at 79.5 ppm. Signals from C-5 of esterified and unesterified α-GalA are at 71.3 and 72.3 ppm, respectively. The signals at 69.0 ppm are from C-2 and C-3 of α-GalA.[25]

Differences between the ^{13}C NMR spectra of 50 °C and 100 °C fractions within each cultivar suggest more neutral side chains in the former. This corresponds well with the results presented in Table 1 and 2, and this was also seen in previous studies of pectin from *Plantago major* where the most active highly branched pectin fraction was isolated from a 50 °C extract.[6]

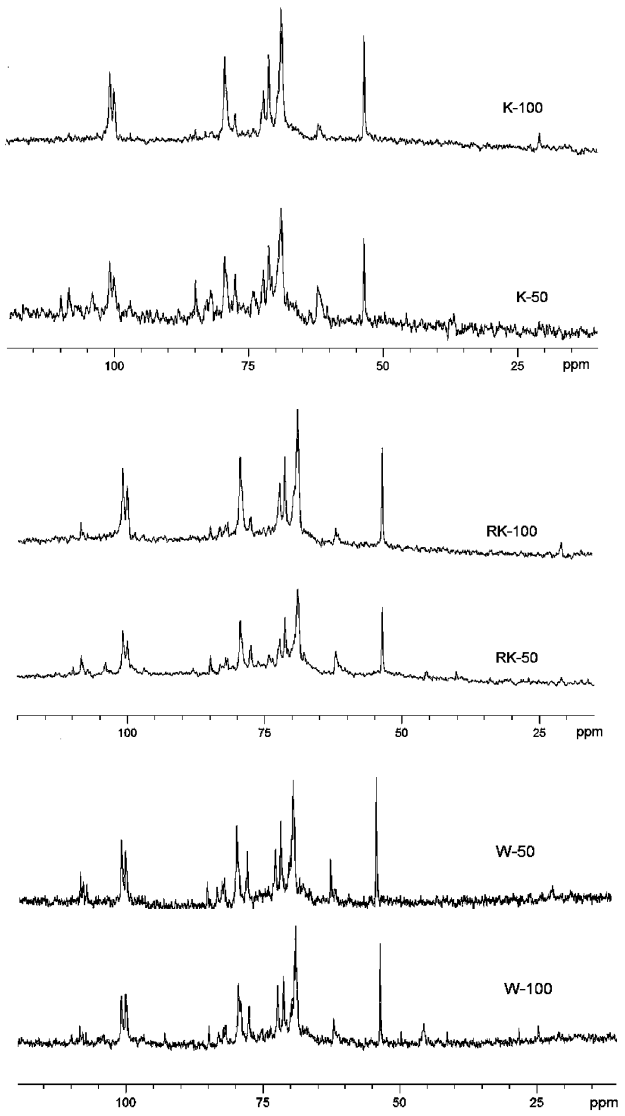

Figure 2. ^{13}C NMR spectra of K-100 (upper spectrum), K-50, RK-100, RK-50, W-50, and W-100.

Structure−Activity Relationships (SAR). Multivariate statistics were used to investigate possible SARs. The measured activity (ICH_{50}) was tested against different analyzed chemical parameters, such as individual monosaccharide levels, total carbohydrate content (T-CHO), detected linkages, phenolic compounds and protein as well as molecular weight distribution M_w, M_n) and polydispersity (M_w/ M_n). Activity versus the average size of side chains (SC) was also studied.

Molecular Weight Distribution, Average Size of Side Chains, Monosaccharides, and Activity. The constituent sugars, their linkages, and the average size of side chains (SC) were found to be significant regarding activity responses whereas M_w, M_n, and M_w/M_n were not. On the basis of PLSR, Gal and GalA were the most significant pectin monosaccharides for prediction of ICH_{50} activity, as presented in Figure 3. The loading plot, with the different analyzed parameters, shows how the analyzed chemical parameters relate to the activity (ICH_{50}). The level of Ara also contributed to the prediction of the ICH_{50} value, however to a lesser degree. With these three sugars and SC, the PLSR method could explain 92% of the variation found in the ICH_{50} values, and the validation of the method showed a correlation of 0.84. The loading plot also indicates that an increased amount of Ara and Gal result in a desired decrease in the ICH_{50} value, i.e., the more Gal and Ara, the higher activity

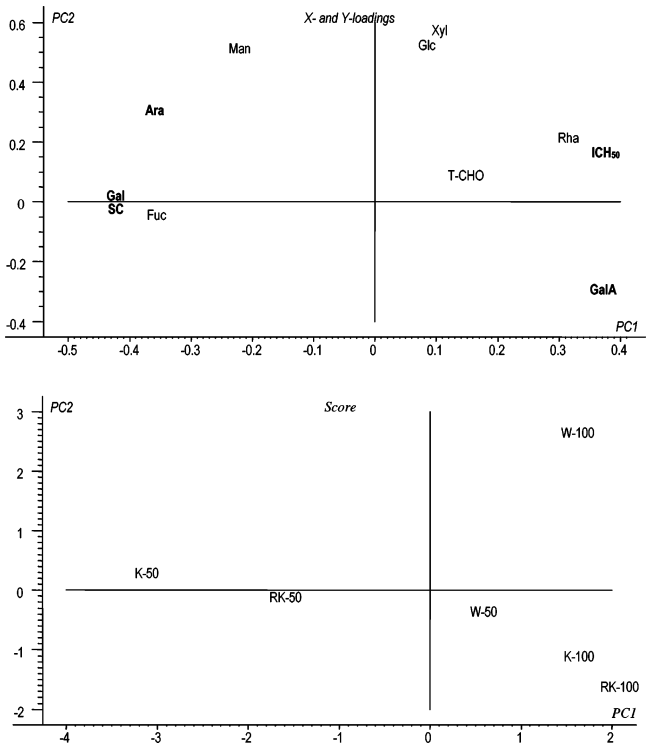

Figure 3. PLSR plots. Upper plot: Loading plot with monosaccharide distribution (*x*-variables) and ICH_{50} values (*y*-variable). Lower plot: Score plot of *Brassica* fractions. T−CHO = total carbohydrate content. SC = average size of side chains determined as (Gal+Ara)/(1→2,4)-Rha*p*. Characters written in bold are significant for the model.

in the complement-fixation test. In contrast, an increase in GalA gives a higher ICH_{50} value. Also the higher the SC, the lower ICH_{50} value, showing that the longer side chains result in more activity, in the fractions tested.

The corresponding score plot shows the distribution of the different samples and how they associate to the different *x*-variables as well as the *y*-variable. The RK-50 and the K-50 is located to the left in the plot, corresponding to low ICH_{50} values and GalA levels. These fractions contain higher amounts of Gal and Ara compared to the other samples. W-50 is located close to the K-100 and RK-100, indicating their similar chemical composition, but different from the RK-50 and K-50 samples. W-100 differs from the other samples extracted at 100 °C due to its high ICH_{50} value and Ara content.

Linkages and Activity. Since the monosaccharide content is related to the activity, it was also interesting to investigate possible linkage-specific activity. A PLSR analysis (Figure 4) was performed to study possible relationships between different linkages detected and the measured activity. (1→3)-linked Fuc and (1→3)-linked Gal, (1→3,6)-linked Gal, and (1→4)-linked GalA were the linkages shown to be most significant for the activity. However, the (1→3) Fuc linkages detected were only found in trace amounts, and this contribution was therefore considered negligible. These few linkages still accounted for as much as 83% of the variation found in the ICH_{50} value, with the validation of the method showing a regression of 0.66 and a calibration correlation 0.83 when including the (1→2,4)-linked GalA and (1→3,4)-linked GalA in the model, more of the variation found in the ICH_{50} value was accounted for (91%), and the validation correlation was improved to 0.75. Furthermore, it is seen that a higher amount of GalA linkages increases the ICH_{50} value, resulting in less activity in the complement-fixation test. On the other hand, the different Gal linkages give a higher activity (i.e., lower ICH_{50} value). The corresponding

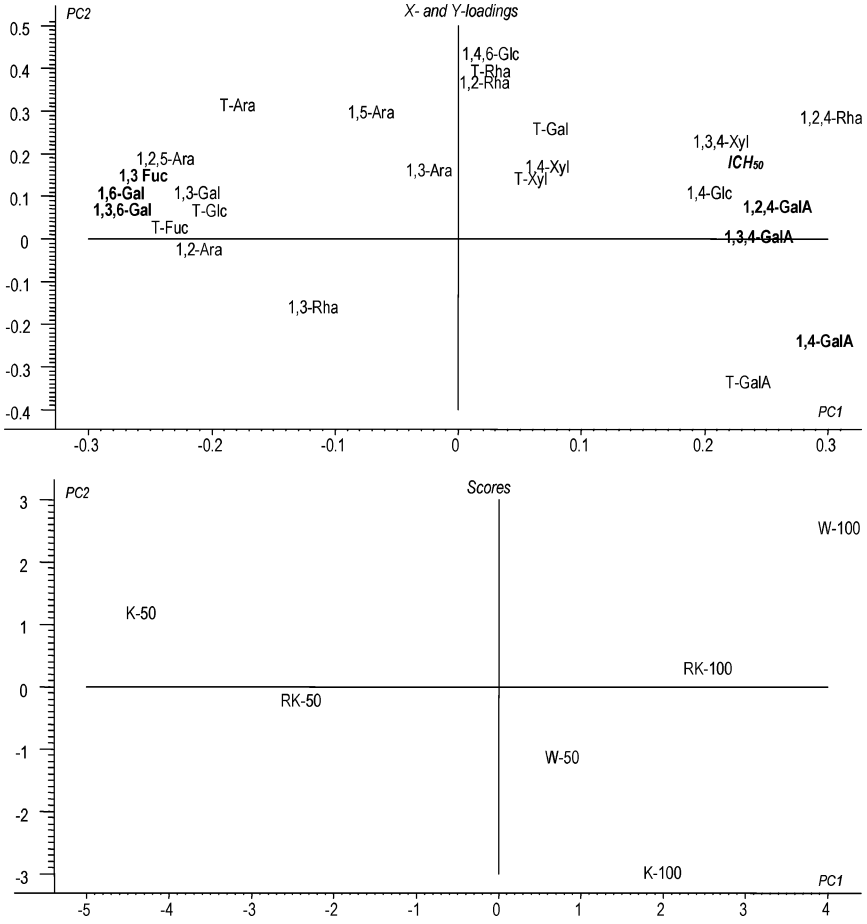

Figure 4. PLSR plots. Upper plot: Loading plot of monosaccharide linkages (*x*-variables) and ICH_{50} value (*y*-variable). Lower: Score plot of *Brassica* fractions. Characters written in bold are significant for the model.

score plot in Figure 4 show that the K-50 and the RK-50 have a higher amount of (1→3)-linked Gal and (1→3,6) Gal linkages than the others, while less content of (1→4)-linked GalA which results in a lower ICH_{50} value and a higher activity. Interestingly, even if the total Ara content was found to be of importance for the activity, the different Ara linkages were not found to be significant in the PLSR plots.

To summarize, a significant negative correlation between Gal and ICH_{50} values ($p = 0.025$) was registered, i.e., the more Gal, the higher the activity in the complement-fixation test. To be more specific, high activity correlates to (1→6)-linked and (1→3,6)-linked Gal residues ($p = 0.029$ and $p = 0.018$, respectively). This correspond well with previous studies such as those on *Plantago major*, revealing that hairy regions of the pectin containing high amounts of (1→6)-, (1→3)- and (1→3,6)-linked Gal residues are the most active part of the polymer.[6] Similar findings are reported from *Centella asiatica*, another plant used for wound healing. Wang et al. conclude that in pectic polysaccharide isolated arabino-3,6-galactan is the major contributor to immune stimulating activity and that the (1→6)- and (1→3)- linked Gal residues play important roles in the activity.[12] Studies on *Angelica acutiloba* suggest that (1→6)-linked Gal residues attached to the rhamnogalacturonan core may be the minimum structural element necessary for complement activating activity.[11,26] Even though statistical analysis shows that high activity is favored by low amounts of GalA and high amounts of Gal, it does not necessarily mean that the most active fractions should be totally free of GalA and be composed of galactan oligomers only. Small amounts of GalA linked to Rha in the backbone of the hairy regions may be necessary for expressing activity, while high amounts are associated with homogalacturonan regions that are known to possess low or negligible activity.[11] Isolation and testing of hairy regions or purified galactan oligosaccharides from the *Brassica* polysaccharides are beyond the scope of the work presented in this article; however, detailed studies of hairy region structures are in progress.

Contribution from Noncarbohydrate Constituents. Interestingly, the statistical analyses also showed a negative correlation between phenolic compounds and the ICH_{50} value ($p = 0.026$). Phenolic compounds may be present as ferulic acid linked to pectic polysaccharides.[10] Previous studies have shown that kale contains chlorogenic acid, caffeic acid, ferulic acid, and *p*-coumaric acid while white cabbage contains much lower amounts of these hydroxycinnamic acids.[27] The actual nature of the phenolics detected in the present polysaccharide fractions and their contribution to the activity remains to be determined.

Conclusion

This study has shown that the three varieties of *Brassica oleracea*, white cabbage, kale, and red kale, contain similar polysaccharide structures that belong to the pectin family. All the pectin fractions are active on the complement system to some extent, but those extracted at low temperature are more active than those extracted at higher temperature. The fraction obtained from kale extracted at 50 °C (K-50) are the most active while RK-50 from red kale are less active though still highly active compared to the other fractions tested. Structural investigations have shown that high amounts of side chains with (1→6)- and (1→3,6)-linked Gal residues correlate to high activity while high amounts of GalA result in less activity in the complement-fixation test.

In a wider perspective, the present study supports the hypothesis that the observed immunological effect may be a general property of pectin polysaccharides that contain hairy regions. However, the magnitude of the activity seems to vary, depending on the pectin source and is influenced by the content of certain side chains in the polymers.

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