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Use of a Plackett−Burman Experimental Design to Examine the Impact of Extraction Parameters on Yields and Compositions of Pectins Extracted from Chicory Roots (*Chicorium intybus* L.)

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Chicory root pectin was isolated by acid extraction followed by alcohol precipitation. Because the extraction conditions have important effects on the features of pectins, an experimental design was used to study the influence of 17 different extraction parameters on yield and composition of pectin: pH, temperature, time of extraction, solid/liquid ratio, and different pretreatments of the pulps before extraction. Twenty extractions were conducted and examined for their significance on yield and sugar content using the Plackett−Burman factorial design. The acid extraction of chicory roots resulted in an average yield of 11% containing 86% of sugars. It was found that extraction temperature, time, protease pretreatment, water purity, and water washing of pulps significantly affected yield and pectin composition with an increase of yield and purity of pectin in harsher extraction conditions.

KEYWORDS: ***Cichorium intybus*; pectins; chicory roots; extraction; Plackett−Burman design; characterization**

INTRODUCTION

Chicory (*Cichorium intybus* L.) is cultivated for its roots, a source of fructan used in food ingredients (*1*, *2*), or in nonfood purposes (*3*). Chicory root pulps and leaves are an important byproduct of the inulin-processing industries and are usually used in cattle feed, fiber production (*4*), or natural antioxidant polyphenols production (*5*). In order to explore the possibilities of valorization of chicory root pulps, the chemical composition and yield of acid-extracted pectins were studied using a Plackett−Burman factorial design (*6*).

Pectins are a family of complex polysaccharides from the cell walls of higher plants and consist of a backbone in which smooth α-D-(1−4)-galacturonan (GalA) regions are interrupted by ramified rhamnogalacturonan regions highly substituted by neutral side chains such as galactan, arabinan, or arabinogalactan (*7*−*11*).

Usually, pectin is obtained by aqueous extraction of raw material under mild acidic or alkaline treatment. The basic extraction process yields a low degree of esterification pectin, whereas pectin with a high degree of esterification is generally obtained by acid extraction. In the acid extraction process, plant material is treated with acid ranging from pH 1.0 to pH 3.0, at temperatures between 70 and 95 °C. The extraction time has to be sufficient for recovering required amounts and quality of pectin from the plant material (*12*, *13*). After filtration, pectin is precipitated from the extraction juice by alcohol precipitation. The precipitated pectins are isolated from the solution by filtration or other separation means; it is then washed, dried, and milled to the desired size.

Previous works reported that temperature, pH, time, washing method, and the extracting agents could modify both the quantity and the quality of several pectins extracted from raw materials such as apple pomace, citrus peels, beet pulps, or sunflower head (*12*−*22*). In order to develop the optimal way for pectin isolation, the influence of extraction parameters (pH, temperature, time, etc.) was investigated by using an experimental design. This statistical approach allowed the quantification of each parameter and their potential interactions on the extraction yield and chemical characteristics of pectins. The first studies on chicory roots were limited to the content and the carbohydrate composition of pectins. Results are mainly compared with other roots largely and more recently studied like sugar beet pectin and are also compared with sunflower because it belongs to the same family of Asteraceae.

MATERIALS AND METHODS

Plackett−Burman Design (PBD). To find the main extraction factors affecting yield and quality of chicory root pectins, a two level PBD was formulated for 17 factors selected from previous studies (**Table 1**). This design was practical, especially when the investigator was faced with a large number of factors and was unsure which settings

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Table 1. PBD ($n = 20$) for the Determination of Significant Variables on Yield and Quality of Chicory Root Pectins

	variables																
run	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+
2	−	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−
3	+	−	+	+	−	−	+	+	+	+	−	+	−	+	−	−	−
4	+	+	−	+	+	−	−	+	+	+	+	−	+	−	+	−	−
5	−	+	+	−	+	+	−	−	+	+	+	+	−	+	−	+	−
6	−	−	+	+	−	+	+	−	−	+	+	+	+	−	+	−	+
7	−	−	−	+	+	−	+	+	−	−	+	+	+	+	−	+	−
8	−	−	−	−	+	+	−	+	+	−	−	+	+	+	+	−	+
9	+	−	−	−	−	+	+	−	+	+	−	−	+	+	+	+	−
10	−	+	−	−	−	−	+	+	−	+	+	−	−	+	+	+	+
11	+	−	+	−	−	−	−	+	+	−	+	+	−	−	+	+	+
12	−	+	−	+	−	−	−	−	+	+	−	+	+	−	−	+	+
13	+	−	+	−	+	−	−	−	−	+	+	−	+	+	−	−	+
14	+	+	−	+	−	+	−	−	−	−	+	+	−	+	+	−	−
15	+	+	+	−	+	−	+	−	−	−	−	+	+	−	+	+	−
16	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	+	+
17	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	+
18	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−
19	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	+
20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Table 2. Low and High Settings for the 17 Examined Factors Under Investigation

variables		low level (−1)	high level (+1)
dry matter	A	fresh pulps	dried pulps
milling	B	not milled	milled
addition of calcium	C	without	with
pH	D	1.5	2
extraction time (h)	E	1	4
extraction temperature (°C)	F	80	94
solid/liquid ratio	G	1/29	1/81
stirring during extraction	H	with	without
pulps washing with water	I	with	without
pulps washing with HCl	J	with	without
protease digestion of pulps	K	no	yes
harvesting date	L	September	December
centrifugation rate (rpm)	M	4000	9000
centrifugation time (min)	N	10	20
ethanol volume	O	1	4
precipitation time (min)	P	20	60
water purity	Q	distilled	tap water

were likely to produce optimal or near optimum responses. A design of a total of 20 experiments was generated, and **Table 2** lists the high (+1) and low (−1) levels of each variable. The 20 experiments were carried out in triplicate.

Materials. Chicory roots from mixed varieties were harvested by Warcoing (Belgium) (in September or in December). Pectins were isolated after inulin extraction from surpressed or dried pulps with a dry matter of 20 and 85%, respectively. Before extraction, pulps were milled using a grinder (IKA, model M20, Germany). The required conditions of extraction were set according to the different values of the PBD. All chemicals used were the purest available. Calcium sulfate dihydrate and pronase for pulps pretreatment; galacturonic acid (GalA), *meta*-hydroxydiphenyl, and sodium tetraborate for galacturonic determination; and rhamnose (Rha) monohydrate, galactose (Gal), glucose (Glu), Ara, xylose (Xyl), 2-methylimidazol, sodium borohydride, and dimethylsulfoxide anhydre for monosaccharide determination were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Other chemicals were purchased from VWR International-Merck-Eurolab (Belgique).

Chicory Roots Pulps Pretreatment. On the basis of previous works (*14*, *23*−*25*), the temperature and pH of the washing medium, washing time, and solid to liquid ratio were considered to be important factors affecting the extraction yield and the quality of sunflower pectin. These pretreatments were applied or not applied following the factor level of the PBD.

Calcium Addition. As the addition of calcium was generally used to firm the tissue (*26*), the natural calcium content of chicory root pulps was increased from 0.7 to 1.0% with $CaCl_2 \cdot 2H_2O$.

Protease Treatment. As in the work of Michel et al. (*13*) and Pagan and Ibarz (*27*), enzymatic digestion by protease was used in order to solubilize proteins before pectin extraction. Pulps were first treated with 100 mM sodium phosphate buffer (pH 7.5) containing 0.01% of pronase at a solid/liquid ratio of 0.85/50. Incubations were performed at 37 °C overnight. Pulps were removed by filtration through Millipore 20 μm nylon filter disc under vacuum and were submitted to washing or to extraction.

Acid Washing. In order to decrease the level of ion exchangeable cations, pulps were washed with 1% hydrochloric acid for 10 min at room temperature and at a solid/liquid ratio of 0.85/10 (*26*). The slurry was filtered through Millipore 20 μm nylon filter disc under vacuum. Pulps were then rinsed with water under vacuum.

Water Washing. Pretreatment with a hot water-washing process was used prior to pectin extraction to improve quality by removing water soluble constituents such as calcium phosphate. Pulps were washed for 20 min at 82 °C in a water bath at a solid/liquid ratio of 1/30. The slurry was filtered through a Millipore 20 μm nylon filter disc under vacuum.

Water Purity. Two water types, namely, tap water and distilled water, were used for all parts of the experiments, and the value was determined by the level value of the PBD. The conductivity of distilled and tap water was about 2 and 985 μs/cm, respectively.

Extraction of Pectins. Details on pretreatment and extraction are given in **Figure 1** with high level values for the 17 selected variables. The two levels of some factor (pH, time, T°) were similar to those selected for sugar beet pulps (*13*, *15*). Chicory root pulps were manually stirred (in a glass reactor), and the pH was adjusted with diluted H_2SO_4. Mineral acid was chosen because it gives higher yields than organic acids (*8*). After extraction for a given period of time (1 or 4 h), at a defined temperature (80 or 94 °C) and with a suitable solid/liquid ratio (1/29 or 1/80), the residue was separated by filtration through a Millipore 20 μm nylon filter. The pectin extract was cooled down for about 30 min in an ice bath and was precipitated by adding 1 or 4 volumes of denatured ethanol for 20 or 60 min at room temperature. Precipitated pectin was recovered by centrifugation at 4000 or 9000 rpm for 10 or 20 min. The precipitate was collected and dispersed in 50 mL of distilled water before freeze drying.

Independently of the PBD, two extractions were also conducted similarly to the run number 9 with different pH, time, and temperature. Dried pulps without pretreatment were suspended in distilled water (s/l ratio 1/81), and the pH was adjusted with dilute H_2SO_4. The extraction A was maintained for 1 h at 80 °C and at pH 2. The extraction B was maintained for 4 h at 95 °C and at pH 1.5. The supernatant was filtered through a Millipore 20 μm nylon filter. The pectin extract was cooled down for about 30 min in an ice bath and was precipitated by adding 4 volumes of denatured ethanol for 60 min at room temperature. Precipitated pectin was recovered by centrifugation at 9000 rpm for 20 min. The precipitate was collected and dispersed in water before freeze drying. Dried pectins were stored until use at room temperature in desiccator with $CaCl_2$ pellets.

Analytical Methods. *Moisture.* The moisture content of the pulps was determined as the weight loss after oven drying at 105 °C for 24 h.

Yield. The extraction yield was calculated based on dry matter (mg/g) of the root pulps before pretreatments. Each extraction was conducted in triplicate.

GalA. The GalA content was determined photometrically from a standard curve of GalA according to the *m*-hydroxybiphenyl method (*28*). It was a simple and indirect method to evaluate the pectin purity. The pectic substance content was expressed as mg GalA per g of dry weight of pectin.

Neutral Sugars. Neutral sugars were analyzed by gas chromatography after sulfuric acid hydrolysis and conversion to alditol acetates according to the procedure implemented by Blakeney et al. (*29*) and modified by Garna et al. (*30*): (i) Hydrolysis: 3 mL of 1 M H_2SO_4 was added to 50 mg of sample and maintained at 100 °C for 3 h. After it was cooled, the hydrolysate was neutralized by 25% NH_4OH. Then, 1 mL of 2-deoxyglucose was added as an internal standard (ii). Reduction: 0.4

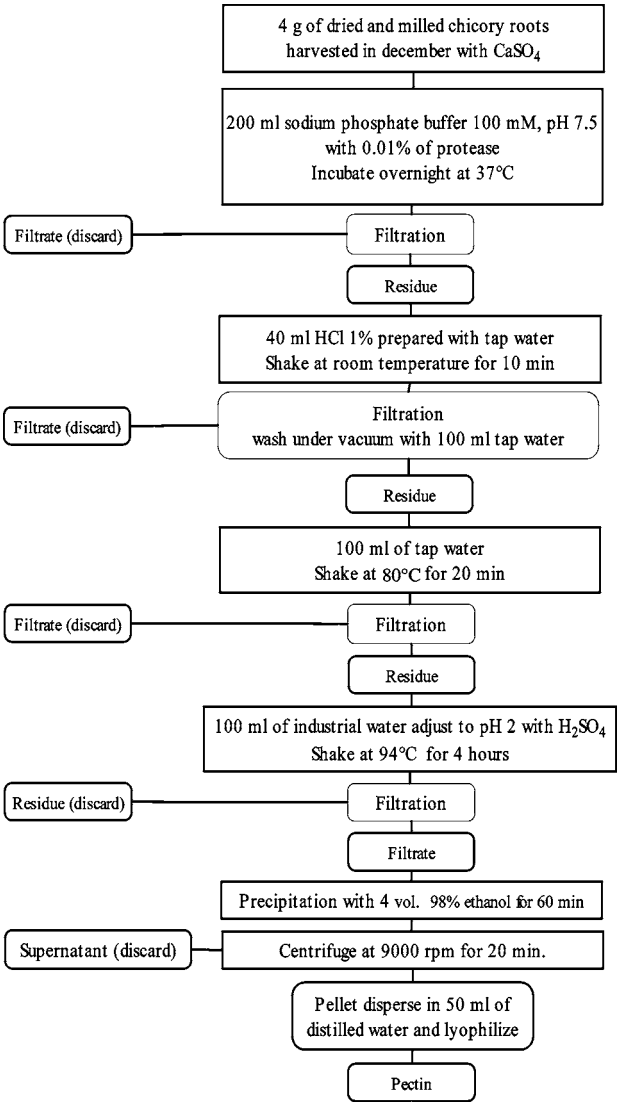

Figure 1. Flow diagram of pectin extraction procedure with high level values for the 17 variables of the PBD.

mL of sample was reduced with 2 mL of a 2% $NaBH_4$ in DMSO and then incubated for at least 90 min at 40 °C. The excess $NaBH_4$ was eliminated by glacial acetic acid (iii). Acetylation: 0.6 mL of acetic anhydride was added followed by 0.4 mL of 1-methylimidazole. Then, 10 mL of water was added to eliminate the excess acetic anhydride. After they were cooled, alditol acetates were recovered in 3 mL of dichloromethane before injection into the chromatograph (HP 6890 GC with a flame ionization detector). The column was a HP-1 (30 m × 0.50 mm, Hewlett Packard Co., Palo Alto, CA). The oven temperature started at 120 °C with a heating rate of 4 °C/min to 220 °C. The injection temperature was 250 °C.

Degree of Methylation (DM) and Degree of Acetylation (DA). Methoxy and acetyl groups were released from pectins by saponification with 0.2 M NaOH in 50% isopropanol for 2 h at 4 °C and quantified by high-performance liquid chromatography (HPLC) on an Aminex HPX-87 column (300 mm × 7.8 mm, BioRad, Hercules, CA) (*31*).

Molecular Weight. The average molar mass was determined by high-performance size exclusion chromatography method on a Waters 2690-HPLC system (Waters Inc., Milford, MA) equipped with TSKgel GPWXL column (300 mm × 7.8 mm, TosoHaas Co. Ltd., Tokyo, Japan) (*32*).

Statistical Analysis. The statistical software used to evaluate the experimental design results was Minitab (version 13.31; Minitab Inc., State College, PA).

RESULTS

No publication exists on chicory root pectins. For this reason, results are mainly compared with other sugar beet pectins. **Table 3** presents the extraction yields, GalA, and sugar contents of different extractions.

Extraction Yield. Pectin yields, expressed as dry weight of the extract, vary from 32 to 242 mg/g of the root pulp dry matter before pretreatments. Our highest yield is similar to the one previously observed from sugar beet pulps (23.7% at pH 1.5/5 h/95 °C) (*13*).

The pareto chart of effects is a useful plot for identifying the factors that are important. In these charts, bar lengths are proportional to the absolute value of the estimated effects, helping to compare their relative importance. Working at a 90% confidence interval determined by a vertical line as critical *P* value ($\alpha = 0.1$), the pareto chart for the yield ratio (**Figure 2**) shows that the significant effects are, ranked by order of importance, the protease digestion, the extraction temperature, the ethanol volume, and the water type, and working at a 85% confidence interval, the effect of the extraction time becomes significant. Others parameters did not play an important role in the extraction yield of pectin.

The Minitab software also provides the main effect plot. This plot reveals the positive or negative effect of a factor from level −1 to level +1 on the extraction yield and chemical composition. **Figure 3** displays the main effect plot of the variables for extraction yield and shows that the pretreatment with a protease negatively affects the extraction yield. The lowest yield observed after incubation with protease may be due to a loss of pectin in the filtrate. The pretreatment conditions (pH 7.5 at 37 °C for 16 h) are favorable to a β-elimination as pointed out earlier (*33*). The presence of pectin can be easily verified by the determination of GalA in the filtrate. An increase of the time (with $\alpha = 0.15$) and temperature improve the recovery of pectin. These observations are in accordance with the studies on pectin extraction from fresh peach pomace (*27*) or from sugar beet pulps (*13*, *14*). The highest yields (pectin and GalA) are obtained, ranked by order of importance for runs 8, 1, and 18. These results are observed with the most aggressive conditions of extractions with 4 h at 94 °C. Because pulp washing decreases yield, pectin recovery was logically lower in runs 1 and 18 as compared to run 8. Moreover, in the same conditions of temperature and time, experiment 5 shows the lowest yield with 32 mg/g as compared to runs 8, 1, and 18. The predigestion step with protease that occurs in run 5 but not in runs 1, 8, and 18 negatively affects the yield as suggested above.

Increasing ethanol from 1 to 4 volumes improves the extraction yield as well. The highest yield was obtained for run with 4 volumes of alcohol (run 8) and the three lowest yields (runs 5, 2, and 20) were observed when pectins were recovered with only 1 volume. Moreover, only 1 volume was also added in run 1 when harsher conditions of extraction were used. Strong conditions of extraction lead to smaller pectin molecules, which require a higher addition of alcohol to be recovered as shown in **Table 4**.

Finally, the use of tap water also positively affects the extraction yield. The marked increase in pectin yield and GalA yield can be attributed to the formation of ionic bonds between calcium or other divalent cations from tap water and exposed carboxyl groups in the C_6 of GalA (*34*, *35*).

Surprisingly, some parameters do not significantly influence the extraction yield. For example, our result shows that the pH does not seem to have a significant effect on the pectin extraction yield whereas Levigne et al. (*14*) concluded that pH significantly

Table 3. Yields (mg/g of Dry Weight) and Sugar Composition (mg/g of Dry Weight) of Pectins Extracted with Conditions Detailed on the PBD

	yield[a] (mg/g)		composition of the pectic extract[a]								
run	pectin	GalA	GalA	Rha	Ara	Xyl	Man	Glu	Gal	neutral	total
1	196 ± 0.8	148	755 ± 1.2	10	7	0	0	2	21	41 ± 0.2	796
2	35 ± 0.3	27	772 ± 0.7	10	8	0	0	1	27	46 ± 0.1	818
3	51 ± 0.7	39	759 ± 0.2	22	16	1	0	3	58	100 ± 0.2	859
4	71 ± 0.7	55	772 ± 0.2	15	14	1	1	3	56	89 ± 0.6	861
5	32 ± 1.2	25	782 ± 0.1	15	10	1	0	2	34	61 ± 0.2	843
6	116 ± 0.4	80	690 ± 0.4	11	12	1	0	1	37	63 ± 0.2	753
7	65 ± 1.1	44	684 ± 1.1	12	12	1	0	2	29	55 ± 0.5	739
8	242 ± 1.0	182	750 ± 0.2	11	8	0	0	0	24	44 ± 0.4	794
9	119 ± 0.4	93	779 ± 0.2	16	7	1	0	2	45	71 ± 1.4	850
10	72 ± 0.2	48	671 ± 0.1	18	18	1	0	3	42	82 ± 1.0	753
11	86 ± 0.1	63	738 ± 0.1	12	10	1	0	1	26	50 ± 0.7	788
12	101 ± 0.4	70	697 ± 0.1	24	15	1	0	2	62	105 ± 0.4	802
13	67 ± 1.2	44	660 ± 0.8	12	5	0	0	2	34	53 ± 0.7	713
14	156 ± 0.2	115	738 ± 0.3	19	11	0	0	2	49	81 ± 0.5	819
15	139 ± 0.3	94	677 ± 0.2	19	15	1	0	3	60	98 ± 0.4	775
16	168 ± 0.7	110	656 ± 0.6	18	15	1	0	2	47	83 ± 0.7	739
17	151 ± 0.6	101	671 ± 0.1	13	10	1	0	2	29	55 ± 0.3	726
18	193 ± 0.2	141	732 ± 0.7	15	15	1	0	3	63	96 ± 0.2	828
19	106 ± 0.1	76	714 ± 0.4	11	12	1	0	2	61	88 ± 0.9	802
20	39 ± 0.4	24	626 ± 0.1	19	13	1	0	2	53	88 ± 0.6	714

[a] Mean ($n = 3$) ± SD. [b] Mean ($n = 6$) ± SD.

affected the pectin extraction yield. This discrepancy is probably due to the narrow range of pH used in the present work [1.5−2 vs 1.5−3 for Levigne et al. (*14*)]. The optimum pH value may also comprise between 1.5 and 2, and the pH was not pointed as significant. The extreme yield values are recorded when extraction is conducted at pH 1.5, but the highest average yield is obtained at pH 2.0. Only on the basis of yield, conditions to extract pectin at pH 1.5 were the most effective for run 8. An increase of yield was also observed when pH increases from 1.20 to 1.40 when pectin was extracted from stored pomace (*36*).

Composition of the Alcohol Insoluble Material. *GalA.* Pectin is mainly composed of GalA (626−782 mg/g). These values of GalA content are quite similar to those observed from sugar beet pulps with ca. 65% (*37*) and with 69.6% (*38*) from fresh sugar beet. Industrial pectins contain at least 65% GalA (w/w) (*39*) and contain only small amounts of neutral sugars (5−10%, w/w) (*10*, *40*−*41*). Only on the basis of high GalA content and yield that were comparable to that of citrus pectin, the production of commercially pectin from chicory roots could be considered.

As shown in **Figure 3**, GalA content is predominantly influenced by the water-washing step of the pulps before extraction whereas these factors were not significant for pectin yield. A higher content of GalA is obtained when distilled water is used for pretreatment and extraction. Washing the pulps as pretreatment with hydrochloric acid also improves the GalA content. Improvement of GalA content shown by **Figures 2** and **3** is not so high when pulps are prewashed with HCl, probably due to a shorter time of incubation and a much lower temperature of reaction (10 min at ambient temperature) as compared to water soak (20 min at 80 °C). These results are consistent with previously published data from sunflower head pectins. Shi et al. (*24*, *25*) show that pretreatment with a hot water-washing process prior to pectin extraction improves pectin quality, and Lin et al. (*23*) show that these pretreatements inactivate some enzymes. **Figure 3** also shows the positive effect of the temperature on the pectin purity. The others factors may be considered unimportant as regards to yield and GalA content. In our work, pH is not a key factor at a confidence interval of 90%, but there is a moderate influence of the pH on GalA content with slightly higher contents at pH 1.5, but when results were expressed in GalA yield, the amount of GalA was higher in pH 2. It contrast with the work of Levigne et al. (*14*), there is a moderate influence of the pH on GalA content with slightly higher contents at pH 3, but GalA yields were higher in pH 1. Nevertheless, our results are in agreement with the work of Kalapathy and Proctor (*27*) for whom acid strength used for extraction does not significantly affect the GalA content of soy hull pectin. Pectin with the highest GalA content is produced in extraction run 5, when the lowest yield is also obtained. Similar results were also obtained by Levigne et al. (*14*).

Total Neutral Sugars. All neutral sugar values are presented on pectin dry weight basis. In this study, the total neutral sugar content varies from 40 to 105 mg/g, a little lower than the values obtained for sugar beet pectin (*14*, *15*) or for apple pectin (*43*). The main neutral sugars are Gal, Rha, and Ara with an average content of 43, 15, and 12 mg/g of dry pectin, respectively. Small amounts of Glu, mannose (Man), and Xyl are also identified and could come from hemicelluloses. The neutral sugar composition and content were comparable to that of commercial pectins such as citrus pectin. Gal is the major neutral sugar present in chicory root pectin, probably from galactan and/or arabinogalactan side chain (*44*).

The carbohydrate content only accounted for 713−861 mg/g. The remainder may consist of methanol, acetic acid (substituents of the pectins), and also proteins, salts, and/or residual water. More aggressive conditions improve the yield and purity of pectin (**Figure 3**). Higher GalA content and lower neutral sugars contents are found for the highest extraction temperature, for the longest extraction time, and the lowest pH value such as runs 8, 1, and 5. The use of tap water negatively affects the purity but induces an increase of pectin yield. Unfortunately, pretreatment such as protease predigestion and water or acid washing results in increased pectin loss but improves the purity.

Individual Neutral Sugars. All of the variables considered for the extraction are not significant at a confidence interval of 0.9 for neutral sugars, but for a $p > 60\%$, some parameters significantly affect the individual sugar content.

The Gal content varies from 21 to 63 mg/g (**Table 3**), which is somewhat lower than those obtained from sugar beet (*14*, *38*, *45*, *46*). The main effects of variables on Gal content are

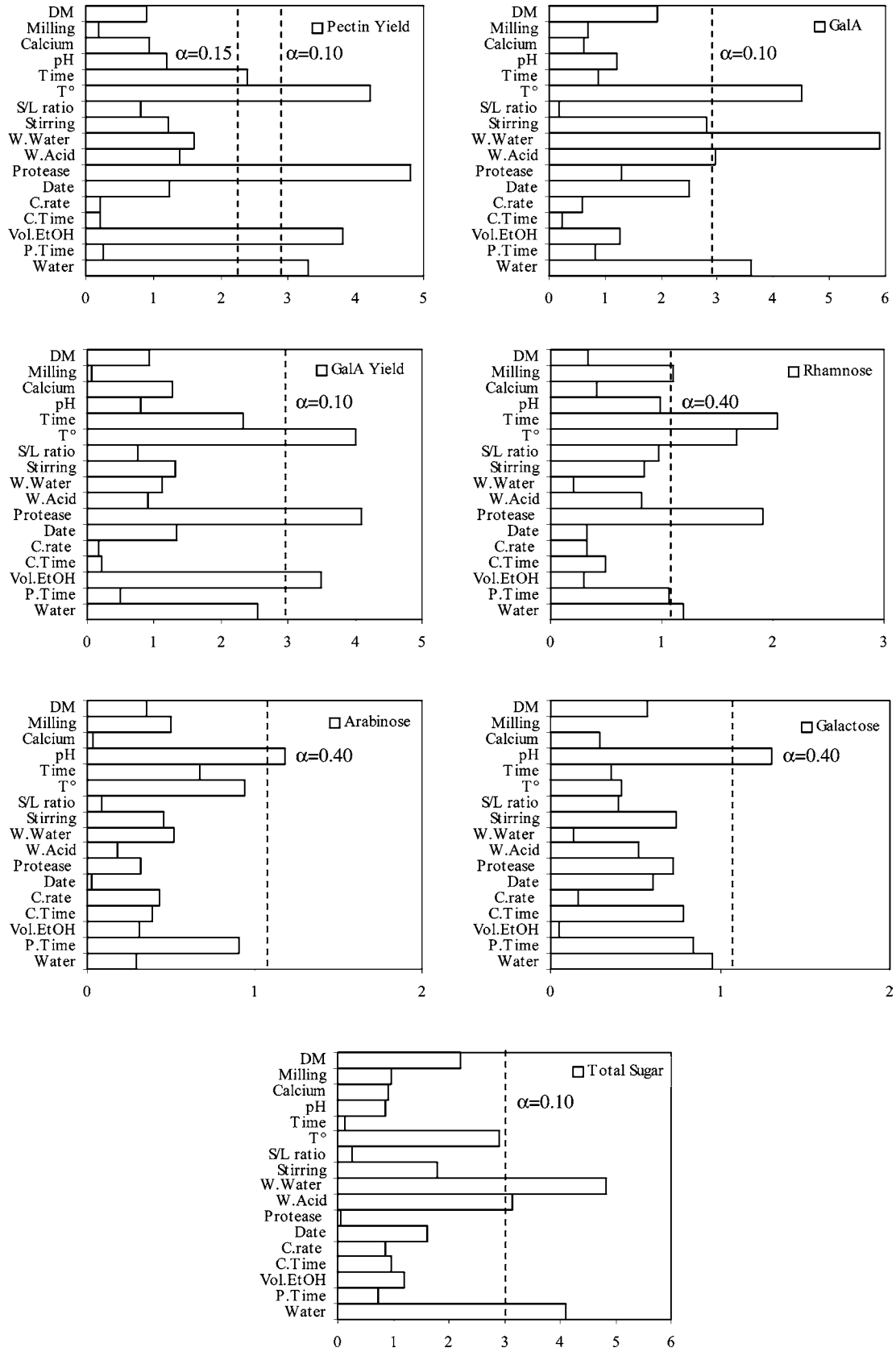

Figure 2. Standardized main effect Pareto charts for the PBD for extraction yield of pectin, for GalA content, and for neutral sugars.

shown in **Figure 2**. The pH is revealed as having a significant effect on Gal content showing that a decrease of pH from 2 to 1.5 induces a decrease of Gal content.

The Rha content varies from 10 to 24 mg/g. The values are lower than those obtained for pectin extracted from sugar beet pulps (*14*, *38*, *45*, *46*). The highest value is observed for run 12 with a content of 24 mg/g. With regard to pareto chart (**Figure 2**), the order of influence on Rha content is the time of extraction, protease pretreatment, temperature, purity of water, and milling. The effect of these factors is highly significant and

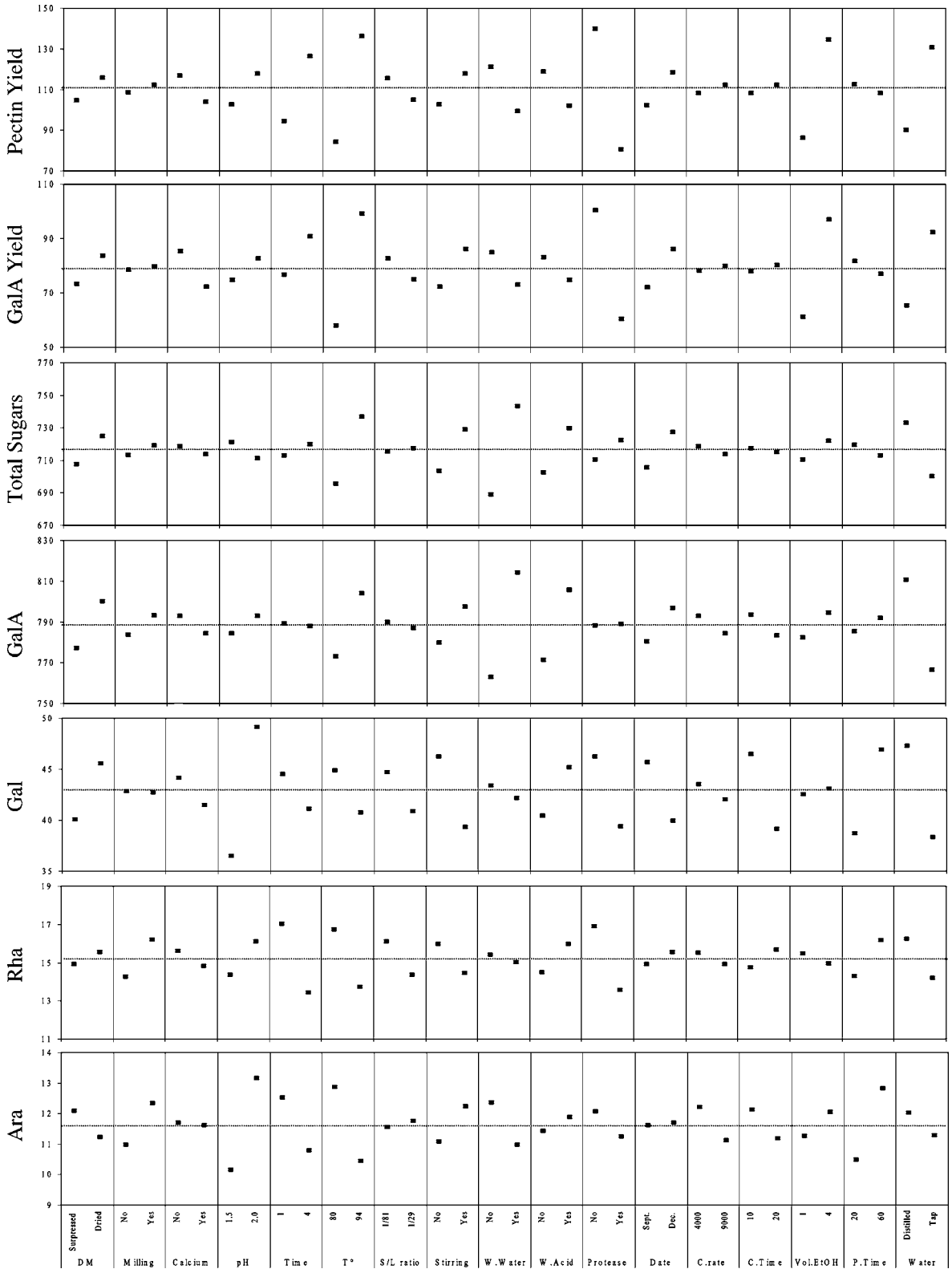

Figure 3. Main effect plots for the GalA, Gal, Rha, and Ara contents and for yield of pectin.

always negative. This shows that Rha recovery decreased when extraction was conducted with pulps, which are submitted to initial proteic digestion and tap water for 4 h at 94 °C.

The GalA/Rha molar ratios comprised between 25 and 65 with an average of 40 are higher than those obtained for sugar beet (*47*) and lemon (*48*) with acidic extraction. This shows that the acid soluble pectin from chicory roots contains lower proportions of rhamnogalacturonic regions than sugar beet or lemon. No factor has a significant influence on this ratio. As opposed to Levigne et al. (*14*), no relation between this ratio and the pH could be highlighted.

The Ara content varies from 5 to 18 mg/g (**Table 3**). The values are lower than those obtained from sugar beet (*14*, *38*, *45*, *46*). The Ara content is mainly affected by the pH: When the pH increases from 1.5 to 2, the recovery of Ara decreases. It is well-known that glycosidic bonds involving the arabino-

Table 4. Yields (mg/g of Dry Weight) and Sugar Composition (mg/g of Dry Weight) of Pectins

		composition of the pectic extract[b]									
run	yield (mg/g)[a]	GalA	Rha	Ara	Xyl	Man	Glu	Gal	DM (%)	DA (%)	MW (kDa)[b]
A	101 ± 1.2	659 ± 0.9	27	11	1	0	2	65	57 ± 2.7	16 ± 2.4	736 ± 2.9
B	231 ± 1.5	787 ± 0.7	10	5	0	0	0	21	21 ± 1.2	5 ± 1.5	274 ± 4.5

[a] Mean ($n = 2$) ± SD. [b] Mean ($n = 4$) ± SD.

furanose moiety are the most acid labile of those commonly encountered in pectins (*49*).

Taking account of all results and in order to confirm our results, two extractions were conducted similarly to conditions in run 9 expect pH, temperature, and time of extraction (**Table 4**). One hour of extraction at 80 °C and at pH 2.0 allows us to extract a pectin with a high molecular weight and a high DM. This pectin can be used as a gelling agent. On the other hand, drastic extraction conditions (4 h, pH 1.5, and 95 °C) lead to a decrease of DM and DA and also a drop of the molecular weight. Pectin obtained by this way could be used as a thickening agent or for the preparation of calcium gels for which pectin with a DM lower than 50 is required.

DISCUSSION

Our first objective was to evaluate the possibility to extract pectin from chicory root pulps. Thus, we focused our research on the purity aspect and on the extraction yield. We wanted to determine the best way to extract pectin and obtain satisfactory yields.

From the above-listed considerations, harsher extraction conditions lead to a higher amount of extracted pectin with higher purity as already shown for sugar beet (*13*, *14*). For example, an increase from the low to the high level for the temperature positively influences yield as well as the GalA content of pectin. Other factors significantly affect the yield and purity of pectin. The water type is an example: the sugar content is lower when pretreatment and extraction are conducted with tap water whereas this factor positively affects the extraction yield. Protease pretreatment only affects the extraction yield. The many extraction parameters probably mask the effect of some factors like the pH and time in this first study. On the basis of the results from the PBD experiment, statistically significant variables (T°, time of extraction) and others variables (pH) with positive effects must be further explored with uniform design experiments to find the optimal value of each factors.

The extraction process is simple and easy to develop on an industrial scale with sulfuric acid if raw material does not contain too much calcium. As there is a correlation between the yield and the quality, the extraction conditions can be selected to achieve a desired quality of pectin. We must take into account the structural features of pectins such as good gelling ability, gel properties, and other physicochemical parameters to choose the most adequate way to extract pectin from chicory roots.

In conclusion, in this present study, the use of a Plackett−Burman experimental design allows the authors to investigate a high number of factors for existence of an impact on yields and chemical composition. It is otherwise a reexamination of pectin extraction conditions on a new material. The statistical methodology is demonstrated to be effective and reliable in selecting the statistically significant factors and finding a first step to optimize the pectin extraction.

A combination of the most effective factors such as high temperature, high time of extraction, and high volume of alcohol for the pectin recovering in the presence of tap water and without protease digestion of the pulp is desirable to achieve high pectin yield with high purity. Using dried and milled pulps harvested in December, stirring the pulps during extraction step, and keeping centrifugation during a higher time at a higher rate also favor high pectin yield. However, the washing steps of the pulps with water and/or with acid and the lower pH value that negatively affect the pectin yield increase the galacturonic content of pectin.

ABBREVIATIONS USED

Ara, arabinose; DA, degree of acetylation; DM, degree of methylation; Gal, galactose; GalA, galacturonic acid; Glu, glucose; Man, mannose; PBD, Plackett−Burman design; Rha, rhamnose; SD, standard deviation; Xyl, xylose.

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