Optimization of pectin extraction from banana peels with citric acid by using response surface methodology

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Abstract

A central composite design was used to determine effects of pH (2.0 – 4.5), extraction temperature (70 – 90℃) and time (120 – 24 0 min) on the yield, degree of methyl esterification (DM) and galacturonic acid content (GA) of pectins extracted from banana peels with citric acid. Changes in composition during the main steps of pectin extraction were followed by Fourier transform infrared (FTIR) spectroscopy. FTIR was also used to determine DM and GA of pectins. Harsh temperature and pH conditions enhanced the extraction yield, but decreased DM. GA presented a maximum value at 83°C, 190 min, and pH 2.7. The yield of galacturonic acid (YGA), which took into account both the extraction yield and the pectin purity, was improved by higher temperature and lower pH values. The optimum extraction conditions, defined as those resulting in a maximum YGA while keeping DM at a minimum of 51%, were: 87℃, 160 min, pH 2.0.

Keywords: polysaccharides; biopolymers; response surface methodology; banana; pectin.

1. Introduction

Bananas (*Musa acuminata,* AAA Group) are one of the most important fruit crops, with a global annual production that surpassed 100 million tons in 2011 (The World Banana Forum, 2014). Bananas are mostly consumed raw, and their processed products include banana flour, chips, and puree (which can be used to produce nectars, smoothies, and a variety of other products). Banana peels constitute about 30% of the fruit, and represent an environmental problem because of their large nitrogen and phosphorus contents as well as their high water content, making them highly susceptible to microbial degradation (González-Montelongo, Lobo, & González, 2010). The use of banana peels as a source of high value compounds such as pectin (Happi Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008b), cellulose nanofibers (Tibolla, Pelissari, & Menegalli, 2014), and phenolic compounds (González-Montelongo et al., 2010; Rebello, Ramos, Pertuzatti, Barcia, Castillo-Muñoz & Hermosín-Gutiérrez, 2014) is interesting not only from an economic point of view, but also from an environmental perspective.

Some previous studies were carried out to investigate effects of process variables on extraction of pectin from banana peels. Qiu, Zhao, Wu, Jiang, Li & Liu (2010) investigated the effects of pH, extraction time, temperature, and salting out time on pectin extraction by using an enzymatic method. Happi Emaga et al. (2008b) evaluated the differences in pH, temperature and time on pectin extraction from banana peels using sulfuric acid. However, no previous report has been found on studying effects of process variables on pectin extraction from banana peels using

45 organic acids, which are more interesting than strong mineral acids from an
46 environmental point of view (Chan & Choo, 2013; Pinheiro et al., 2008). Moreover,
47 because of their lower dissociation constant, organic acids have a lower hydrolyzing
48 capacity than mineral acids, so a lower de-polymerizing effect is expected from citric
49 acid when compared to strong mineral acids (Kermani et al., 2014). Pectin yields
50 from extraction of apple pomace, cocoa husks, and passion fruit peel with citric acid
51 were found to be similar to those obtained with hydrochloric acid (Canteri-Schemin,
52 Fertonani, Waszczynskyj, and Wosiacki, 2005; Chan & Choo, 2013; Kliemann et al.,
53 2009).

54 The objective of this study was to evaluate the influence of pH, temperature, and
55 time on pectin extraction from banana peels with citric acid.

56 2. Materials and Methods

57 2.1. Chemical composition of banana peel powder and changes in FTIR
58 spectra

59 Banana peels at ripening stage 6 (fully ripe bananas, ready for consumption) were
60 collected from Cavendish bananas purchased in the local market (Norwich, UK). The
61 peels were immersed in sodium metabisulfite solution (1% w/v) for 24 h, oven-dried
62 at 60℃ for 24 h, and milled to 0.5 mm in a Retsch Br inkmann ZM-1 centrifugal
63 grinding mill (Retsch GmbH, Haan, Germany).

64 The ash, extractive, and Klason lignin contents of the banana peel powder were
65 determined according to methods TAPPI t413 OM-93 (TAPPI, 1993), TAPPI T204

66 cm-97 (TAPPI, 1997), and TAPPI T222 om-22 (TAPPI, 2000), respectively.
67 Hemicellulose and α-cellulose contents were analyzed as described by TAPPI
68 T203cm-99 (TAPPI, 2009), and holocellulose, according to Yokoyama, Kadla, and
69 Chang (2002).

70 Fourier Transform Infrared (FTIR) spectra were collected from the banana peel
71 powder, the alcohol insoluble residue (AIR), and pectin, in the frequency range of
72 4000-800 cm^{-1} (128 scans at 2 cm^{-1} resolution) on a Nicolet Magna-IR 860 FTIR
73 spectrometer (Thermo Nicolet, Madison, WI, USA). Samples were placed on a
74 GoldenGate diamond ATR accessory (Specac, Orpington, Kent). The empty crystal
75 was used as reference. The spectra were compared in order to understand the
76 changes that occurred during the pectin extraction process.

77 **2.2. Preparation of the alcohol-insoluble residue (AIR)**

78 The pectin extraction follows a preparation of an alcohol insoluble residue, in order to
79 remove low molecular weight compounds, including traces of free galacturonic acid
80 (Happi Emaga et al., 2008b).

81 The alcohol insoluble residue (AIR) preparation was based on the method by
82 Waldron & Selvendran (1990), with some modifications. 100 g of milled peels were
83 washed three times with ethanol, to remove any alcohol soluble phenolics (the first
84 time with 600 mL of boiling 70% v/v ethanol solution for 5 min, the second time with
85 600 mL of boiling absolute ethanol for 5 min, the third time with 600 mL of absolute
86 ethanol at room temperature for 5 min), then washed in 200 mL acetone. Between
87 washings, the material was filtered through a 10 µm nylon mesh. The AIR was air
88 dried at room temperature.

89 **2.3. Pectin extraction**

90 The pectin was extracted from the AIR with citric acid solution (AIR: citric acid
91 solution ratio, 1:20 w/v), according to a central composite design (CCD) with 3
92 variables: pH of the citric acid solution, temperature and time of extraction (Table 1).
93 The extraction was carried out in a water bath under stirring (150 rpm). After
94 centrifugation (1147 g, 30 min, 10℃), the supernata nt was vacuum filtered, added to
95 the same volume of absolute ethanol, and the pH was adjusted to 3.5 (pH of
96 minimum pectin solubility) with KOH. The mixture was stirred for 30 min, left to
97 precipitate at 4℃ for 2 h, and centrifuged (15 min, 4℃, 3500 rpm). The pellet was
98 collected, washed with ethanol 70% (v/v), centrifuged again (20 min, 4℃, 3500 rpm),
99 and dried at room temperature. It was then diluted/dispersed in water (Ystral, 20
100 min), had its pH adjusted to 7, and was again dried and milled to a fine powder using
101 a basic mill (A10, IKA GmbH, Germany).

102 **2.4. Pectin characterization and statistical analyses**

103 The degree of methyl esterification (DM) and the galacturonic acid content (GA) of
104 the banana peel pectin were determined from FTIR spectra (collected as previously
105 described). The DM was determined as described by Manrique & Lajolo (2002), with
106 some modifications. Pectin standards with known DM values of 31% (P-9311), 67%
107 (P-9436), and 89% (P-9561) were obtained from Sigma (Steinheim, Germany), their
108 FTIR spectra were recorded (in triplicates), as well as the spectra of four other
109 samples obtained from blends of the pectin standards (with DM values of 40, 49, 60,
110 and 78). The DM determination was based on the band areas at 1700-1750 cm^{-1}
111 (methyl esterified uronic acids, EUA) and 1600-1630 cm^{-1} (free uronic acids, FUA),

112 calculated by using Origin Pro 9, multiple peak fit function, Gaussian fitting
113 (OriginLab, Northampton, USA). A calibration curve was obtained by plotting DM
114 against the ratio between the EUA peak area over the sum of the EUA and FUA
115 peak areas of the standards, and used to determine the DM of the banana peel
116 pectin.

117 The determination of the galacturonic acid content (GA) was modified from the
118 method described by Monsoor, Kalapathy, & Proctor (2001). A set of 10 calibration
119 galacturonic acid standards was prepared by blending polygalacturonic acid (95%,
120 81325, Fluka) with cellulose (Sigmacell type 20, Sigma) to obtain standards with
121 galacturonic acid contents of 0% (pure cellulose) to 90% (w/w) galacturonic acid.
122 Peak areas were measured as the area above the baseline between 1840 cm^{-1} and
123 1550 cm^{-1}, which was used to calculate the total carbonyl peak area (Monsoor,
124 Kalapathy, & Proctor, 2001). A calibration curve was obtained by plotting GA against
125 the total carbonyl peak area, and used to determine the GA of the banana peel
126 pectin.

127 The yield of galacturonic acid (YGA), which was calculated as

128 $$YGA\ (\%) = \frac{Yield(\%) \times GA(\%)}{100}, \quad (1)$$

129 was considered as a good measure of the performance of the extraction process,
130 since it took into account both the extraction yield, but also the purity of the material
131 extracted.

132 Results of the extraction yield, degree of methyl esterification, galacturonic acid
133 content and yield of galacturonic acid were analyzed using the software Minitab® 16

134 (Minitab Inc., State College, PA, USA). The regressions to represent the responses
135 were obtained and evaluated in terms of their determination coefficients (R^2 values)
136 and the significance of their F values.

137 3. Results and Discussion

138 3.1. Chemical composition of banana peel powder and changes in FTIR
139 spectra

140 The banana peel powder has less than 40% holocellulose, 48% of it being α-
141 cellulose (Table 2), and the remaining 52% being hemicelluloses and pectin. When
142 compared to the values reported by Oberoi, Vadlani, Saida, Bansal, & Hughes
143 (2011) for composition of banana peels, the carbohydrate contents reported in this
144 study were lower, while the extractives content was similar, and the ash and lignin
145 contents were higher (although Oberoi et al., 2011 have quantified only the acid
146 detergent lignin). Moreover, Oberoi et al. (2011) only mentioned the species *Musa*
147 acuminate, but they did not mention which banana cultivar group they used.

148 FTIR spectra of banana peel powder (BPP) was quite similar to the spectra reported
149 by Memon, Memon, Bhanger, Memon, El-Turki, & Allen (2008). Some bands are
150 related to the presence of cellulose, and decreased in isolated pectin (CH_2 bending
151 bands at 1433, 1371, and 1322 cm^{-1}). The AIR presented some bands with
152 decreased intensity when compared to BPP, such as those at 2920 cm^{-1} (C-H
153 stretching vibration) and 2852 cm^{-1} (methoxyl groups), and are ascribed to removal
154 of lipid compounds and part of lignin fractions. The band at 2920 cm^{-1} were further
155 decreased in isolated pectins, which may be attributed to removal of other

polysaccharides (cellulose and hemicelluloses) and lignin. Some lignin-related bands also disappeared (or decreased drastically) in pectin, such as at 2852 cm^{-1} and the shoulder at 1520 cm^{-1} (aromatic skeletal vibration). The peak around 1610 cm^{-1} appears in BPP and AIR as a superposition of C=O stretching vibration of nonesterified carboxyl groups (pectin) with C=C aromatic skeleton stretching (lignin fractions), and decreases in isolated pectin, because of lignin removal. The band at 1737 cm^{-1}, ascribed to the C=O stretching of methyl esterified uronic carboxyl groups, is more intense and shifted to a lower wavenumber in isolated pectin. The shifting is attributed to different absorption between bonded and free polysaccharides, as in banana peel powder and isolated pectins respectively. Some bands are more intense in isolated pectin, such as at 1218 cm^{-1} (-CH_3CO stretching), 1097 cm^{-1} and 1023 cm^{-1} (C-O and C–H stretching), 965 cm^{-1} (C-O bending), 920 cm^{-1} (rocking mode of –CH_3), and 889 cm^{-1} (-CCH and –COH bending at the C-6 position).

3.3. Characterization of extracted pectins and statistical analyses

The calibration curves generated for the degree of methyl esterification (DM) and galacturonic acid content (GA) are represented by Equations (2) and (4), respectively, from which the DM and GA values for all treatments were calculated.

$$DM = 126.3R_A + 2.493 \quad (R^2 = 0.974) \tag{2}$$

$$R_A = \frac{A_{EUA}}{A_{EUA}+A_{FUA}} \tag{3}$$

DM: degree of methoxyl esterification; A_{EUA}: area corresponding to the peak at 1700-1750 cm^{-1} (methyl esterified uronic acids); A_{FUA}: area corresponding to the peak at 1600-1630 cm^{-1} (free uronic acids).

$$GA(\%) = 0.7934.A_{1550-1840} - 0.1985 \ (R^2 = 0.951) \quad (4)$$

GA: galacturonic acid content; $A_{1550\text{-}1840}$: area above the baseline between 1840 cm^{-1} and 1550 cm^{-1}, corresponding to the total carbonyl peak area.

Table 3 presents the experimental responses for all the treatments described in Table 1. Figures 2-5 represent the contour plots for the experimental responses, whose regression equations and statistical parameters are presented in Table 4.

The extraction yield values ranged from 5.2 to 12.2% (w/w, based on dry weight of AIR), within the range reported by Happi Emaga et al. (2008b). The yield was increased by increasing temperatures and lowering pH (Figure 2, Table 4), while the time did not have a significant impact (Table 4). The effects of pH and temperature were similar to those described by Happi Emaga et al. (2008b), although those authors have reported that time had a positive (and highly significant) effect on yield. Other studies (Garna et al., 2007; Masmoudi et al., 2008; Wai, Alkharkhi, & Easa, 2010) also demonstrated that harsh conditions favored pectin extraction yield from apple pomace, lemon by-product, and durian rind, respectively.

DM values from 43.5% to 79% have been obtained, a range similar to that reported by Happi Emaga et al. (2008b) when extracting pectin from banana peels with sulfuric acid, although higher values were expected in this study because of the lower hydrolyzing capacity of citric acid (Kermani et al., 2014). In another study, the same group (Happi Emaga, Robert, Ronkart, Wathelet, & Paquot, 2008a) reported

199 the extraction of low- to high-methoxyl pectins from banana peels (depending on the
200 ripening stage) when using hydrochloric acid as an extractant. Lower temperatures
201 and times and higher pH values produced pectins with higher methyl esterification
202 (Figure 3, Table 4), corroborating findings by Garna et al. (2007), Happi Emaga et al.
203 (2008b), and Wai et al. (2010) for apple pomace, banana peels, and durian rinds,
204 respectively. This is ascribed to the higher de-esterification of the polygalacturonic
205 chain at harsh conditions of temperature and pH (Mort, Qiu, & Maness, 1993).

206 The galacturonic acid contents (GA) in the extracted pectins ranged from 53.1 to 86
207 g/100g, higher than the values reported by Happi Emaga et al. (2008b). Figure 4
208 indicates that GA was enhanced by increasing temperatures and times and
209 decreasing pH values up to an inflection point of maximum GA (about 83˚C, 190 min,
210 pH 2.7, as assessed by the Response Optimizer function of Minitab), beyond which
211 the galacturonic acid contents were rather decreased. The existence of this inflection
212 point is related to the significant quadratic terms of the GA model (Table 4). This
213 behaviour (increasing GA up to a maximum point, followed by decreasing GA) may
214 be due to the combination of concurrent phenomena occurring during pectin
215 extraction with acid at high temperature: the release of sugars as a product of pectin
216 hydrolysis (which contributes to GA), and their degradation by acid and heat (Garna,
217 Mabon, Wathelet, & Paquot, 2004). Indeed, different studies have found different
218 behaviors for GA as a function of extraction conditions, probably because of the
219 predominance of one or the other of those two phenomena. Happi Emaga, Garna,
220 Paquot, & Deleu (2012) and Garna et al. (2007) reported an increased GA with
221 increasing extraction time and temperature. Chan & Choo (2013) found also that an
222 increasing extraction time resulted in higher uronic acid, while an increasing

223 temperature resulted in decreased GA. A very low pH (1.5) was demonstrated by
224 Garna et al. (2007) to result in higher extraction of low molecular weight compounds
225 (nonpectic substances or degraded fractions from pectins) from apple pomace when
226 compared to pH 2.0, i.e., the purity of extracts was impaired by too acidic conditions.

227 The yield of galacturonic acid (YGA) was improved by higher temperature and lower
228 pH values (Figure 5, Table 3), while the remaining terms were not significant (Table
229 4). In order to optimize the extraction, the Response Optimizer function of Minitab
230 was used to define the conditions which produced a maximum YGA while keeping
231 the degree of methoxylation high enough (minimum DM of 51%). Those conditions
232 were defined as 87℃, 160 min, pH 2.0. The suitabilit y of the models to predict the
233 responses was tested by performing the pectin extraction under the selected
234 optimum conditions. The experimental values were found to be in agreement with
235 the predicted ones (Table 5).

236 4. Conclusions

237 Pectins were successfully extracted from banana peels with citric acid, under
238 different conditions of pH, temperature and extraction time. Harsh temperature and
239 pH conditions resulted in higher extraction yield, but at the cost of decreasing the
240 degree of methyl esterification from a maximum of 79% to a minimum of 43%. The
241 optimum conditions of pectin extraction, defined as those which produced a
242 maximum yield of galacturonic acid while keeping a degree of methoxylation of at
243 least 51%, were: 87℃, 160 min, pH 2.0.

5. Acknowledgements

The authors gratefully acknowledge the financial support of the UK Biotechnology & Biological Sciences Research Council (BBSRC) and the Brazilian Agricultural Research Corporation (Embrapa). Authors also thank the National Counsel of Technological and Scientific Development (CNPq, Brazil) for the Research Productivity Fellowship (304179/2012-4) granted to H.M.C. Azeredo, and for the scholarships granted to T.I.S. Oliveira (228859/2013-1) and P.H.F. Pereira (243244/2013-4).

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334 **Figure captions:**
335

336 **Figure 1.** FTIR spectra of banana peel powder, alcohol insoluble residue (AIR), and

337 pectin obtained at 80˚C, 180 min, pH 3.25 (treatment 15).

338 **Figure 2.** Yield of pectin extraction from banana peels.

339 **Figure 3.** Degree of methyl esterification of pectins from banana peels.

340 **Figure 4.** Galacturonic acid contents of pectins extracted from banana peels.

341 **Figure 5.** Yields of galacturonic acid from pectin extraction from banana peels.

Table 1. Experimental conditions of pectin extraction with citric acid, with coded values according to the CCD design, and corresponding real (experimental) values.

Treatment	Independent variables					
	Temperature		Time		pH	
	Coded	Real (˚C)	Coded	Real (min)	Coded	Real
1	-1	74	-1	144	-1	2.51
2	1	86	-1	144	-1	2.51
3	-1	74	1	216	-1	2.51
4	1	86	1	216	-1	2.51
5	-1	74	-1	144	1	3.99
6	1	86	-1	144	1	3.99
7	-1	74	1	216	1	3.99
8	1	86	1	216	1	3.99
9	-1.68	70	0	180	0	3.25
10	1.68	90	0	180	0	3.25
11	0	80	-1.68	120	0	3.25
12	0	80	1.68	240	0	3.25
13	0	80	0	180	-1.68	2.00
14	0	80	0	180	1.68	4.50
15	0	80	0	180	0	3.25
16	0	80	0	180	0	3.25
17	0	80	0	180	0	3.25

Table 2. Composition of banana peel powder on dry matter basis.

Component	Content (g/100 g)
Ashes	11.54 ± 0.19
Extractives	30.65 ± 3.83
Klason lignin	16.77 ± 4.61
Holocellulose	39.02 ± 6.63
α-cellulose	18.74 ± 3.18
Hemicelluloses + pectins	20.28 ± 7.35

Values presented are mean ± standard error for n = 3.

Table 3. Experimental responses for the 17 pectin extraction treatments.

Treatments	Yield (%)	Degree of methylation (%)	Galacturonic acid content (g/100 g)	Yield of galacturonic acid (g/100 g AIR)
1	7.86	70.54	56.86	4.47
2	12.21	60.38	77.74	9.50
3	7.71	68.75	70.17	5.41
4	11.21	43.45	85.99	9.64
5	5.56	76.08	53.08	2.95
6	9.14	65.42	64.60	5.91
7	5.86	76.61	62.18	3.64
8	9.86	51.97	68.01	6.70
9	5.21	78.96	62.93	3.28
10	10.36	61.84	73.95	7.66
11	7.13	69.74	66.70	4.76
12	7.41	71.03	72.77	5.40
13	10.86	62.24	77.80	8.45
14	5.21	68.00	68.89	3.59
15	8.14	70.78	82.65	6.73
16	6.21	69.74	83.46	5.19
17	7.14	72.71	85.75	6.13

Table 4. Regression equations (for the coded variables) and statistical parameters of the models.

Equations	F	R^2
Yield = **7.11 + 1.76 x** + 0.03 y – **1.32 z** + 0.43 x^2 + 0.25 y^2 + 0.52 z^2 – 0.06 xy – 0.03 xz + 0.27 yz	8.25 (p<0.01)	0.91
DM = **71.35** – **7.29 x** – 2.16 y + 2.68 z – 1.20 x^2 – 1.21 y^2 – 3.07 z^2 – 3.64 xy + 0.02 xz + 0.73 yz	4.70 (p = 0.027)	0.86
GA = **84.11 + 5.32 x + 3.24 y – 4.24 z – 6.01 x^2 – 5.55 y^2 – 4.28 z^2** – 1.34 xy – 2.42 xz – 1.13 yz	10.59 (p<0.01)	0.93
YGA = **5.98 + 1.66 x** + 0.27 y – **1.32 z** - 0.07 x^2 - 0.21 y^2 + 0.12 z^2 – 0.09 xy – 0.41 xz + 0.05 yz	13.70 (p<0.01)	0.95

DM: degree of methyl esterification; GA: galacturonic acid content; YGA: yield of galacturonic acid; x: temperature; y: time; z: pH. x, y, and z: coded values ranging from -1.68 to 1.68, according to Table 1. Regression terms in bold were significant (p<0.05).

Table 5. Predicted and experimental response values at optimum conditions (87°C, 160 min, pH 2.0).

Response	Predicted value	Experimental value*
Yield (g/100 g AIR)	13.89	14.23 ± 1.19
DM (%)	51.79	53.73 ± 2.89
GA (g/100 g)	78.12	75.91 ± 4.17
YGA (g/100 g AIR)	11.07	10.81 ± 1.16

DM: degree of methyl esterification; GA: galacturonic acid content; YGA: yield of galacturonic acid. * Mean ± standard deviation for 3 repetitions.

Figure 1 (B&W)

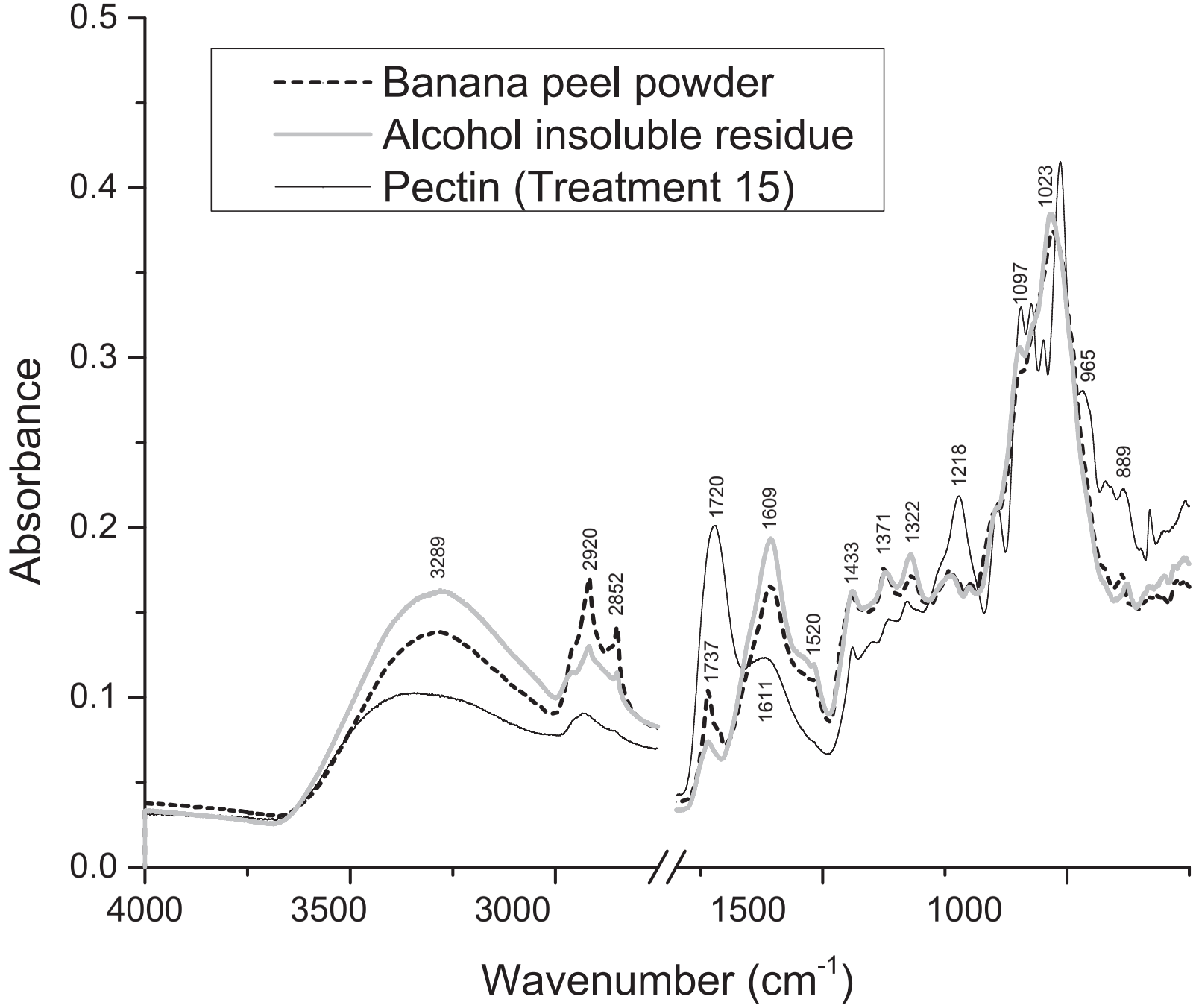
Banana peel powder
Alcohol insoluble residue
Pectin (Treatment 15)
Absorbance
0.0
0.1
0.2
0.3
0.4
0.5
4000
3500
3000
1500
1000
Wavenumber (cm-1)
3289
2920
2852
1737
1720
1611
1609
1520
1433
1371
1322
1218
1097
1023
965
889

Figure 1 (colors)

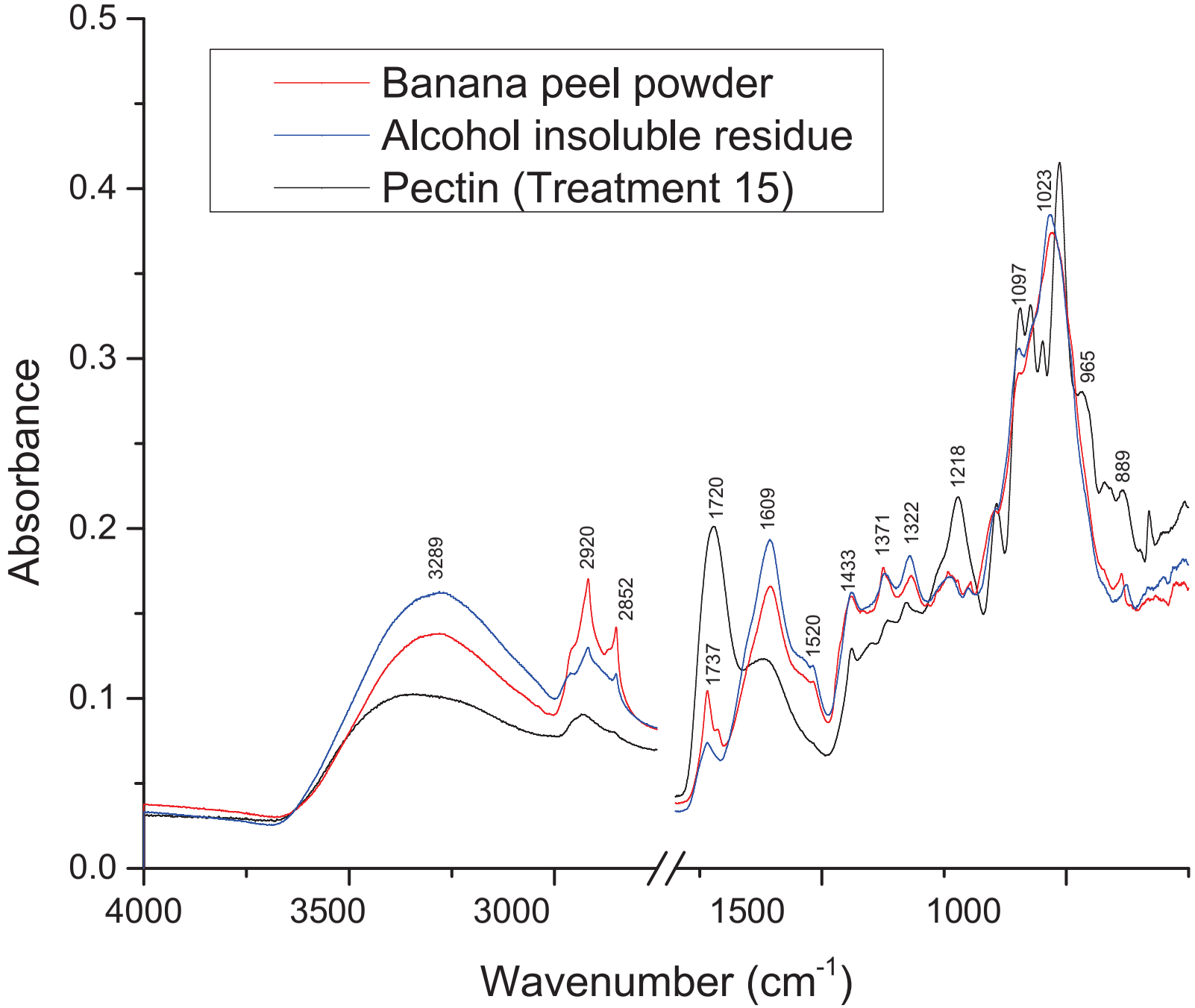

Figure 2 (B&W)

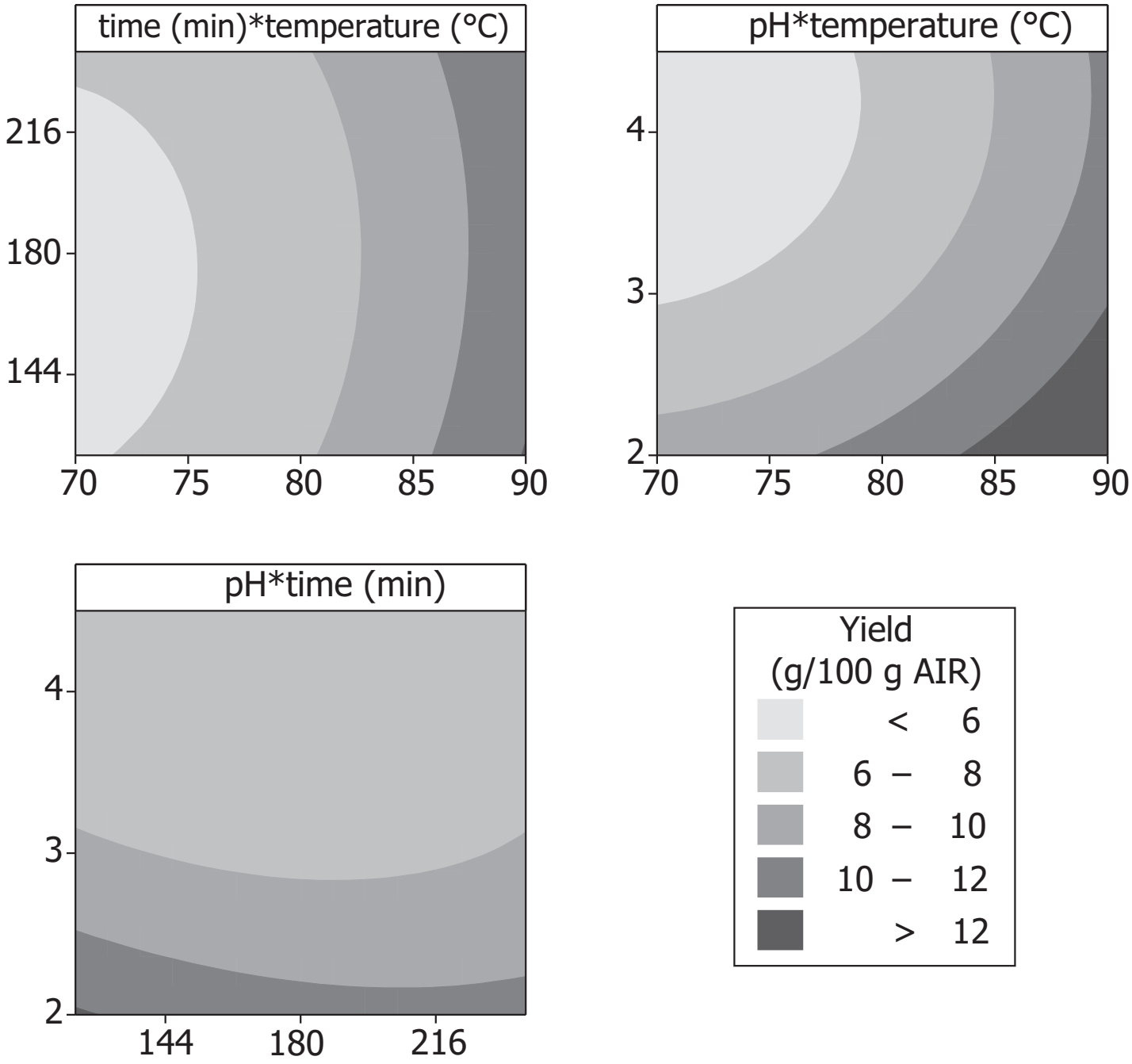
time (min)*temperature (°C)
216
180
144
70
75
80
85
90
pH*temperature (°C)
4
3
2
70
75
80
85
90
pH*time (min)
4
3
2
144
180
216
Yield
(g/100 g AIR)
< 6
6 – 8
8 – 10
10 – 12
> 12

Figure 2 (colors)

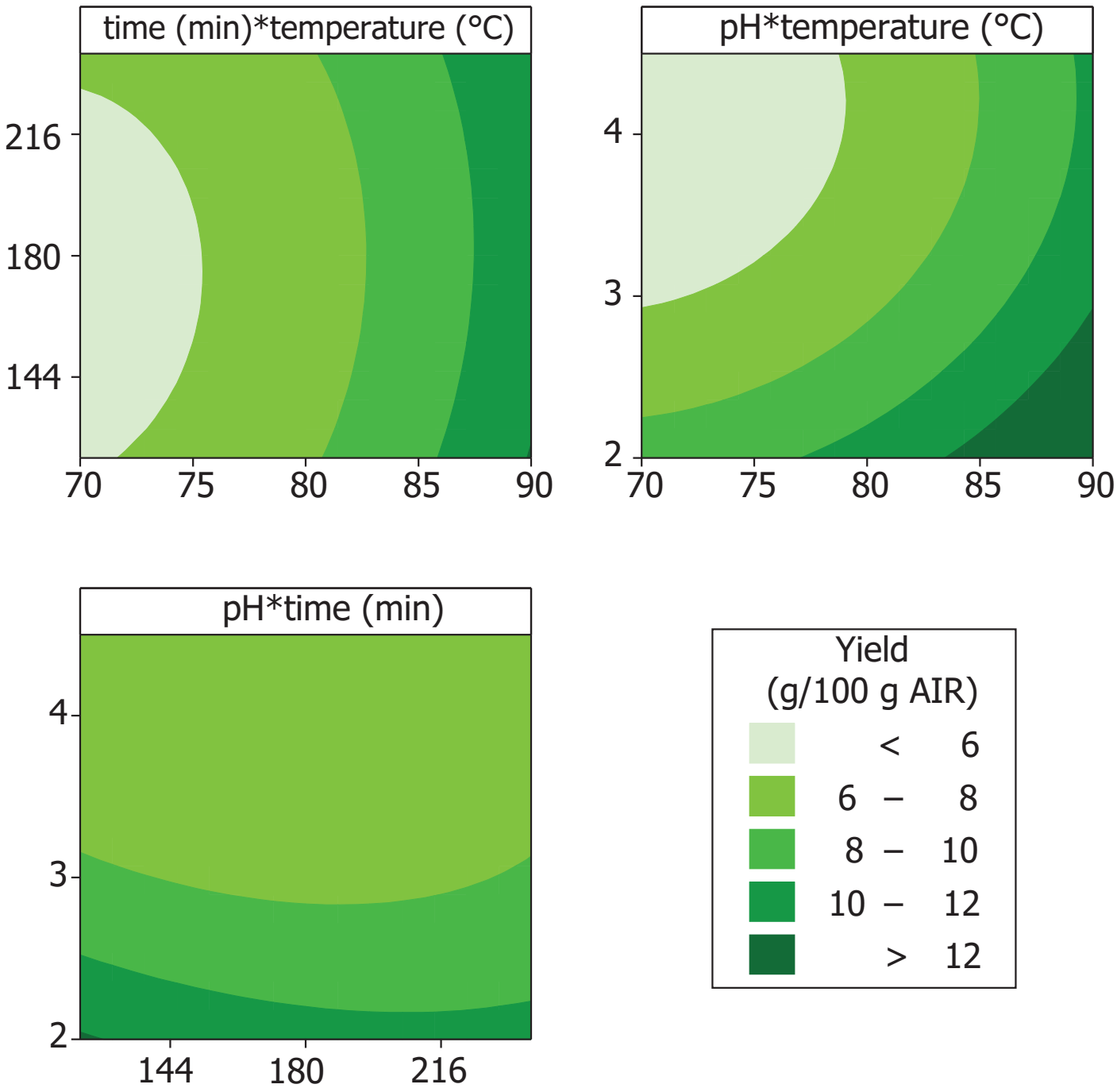

Figure 3 (B&W)

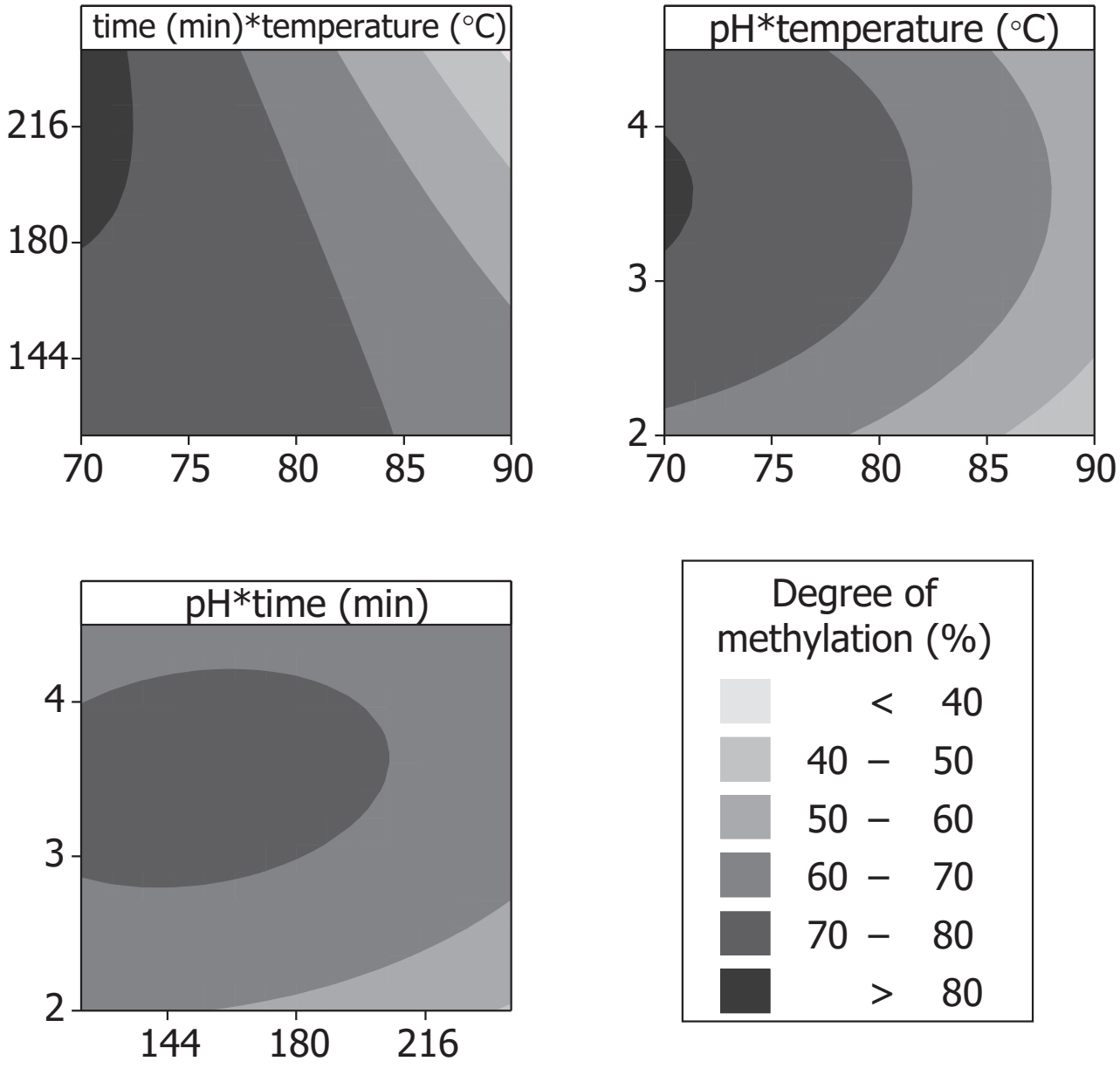
time (min)*temperature (°C)
216
180
144
70
75
80
85
90
pH*temperature (°C)
4
3
2
70
75
80
85
90
pH*time (min)
4
3
2
144
180
216
Degree of methylation (%)
< 40
40 – 50
50 – 60
60 – 70
70 – 80
> 80

Figure 3 (colors)

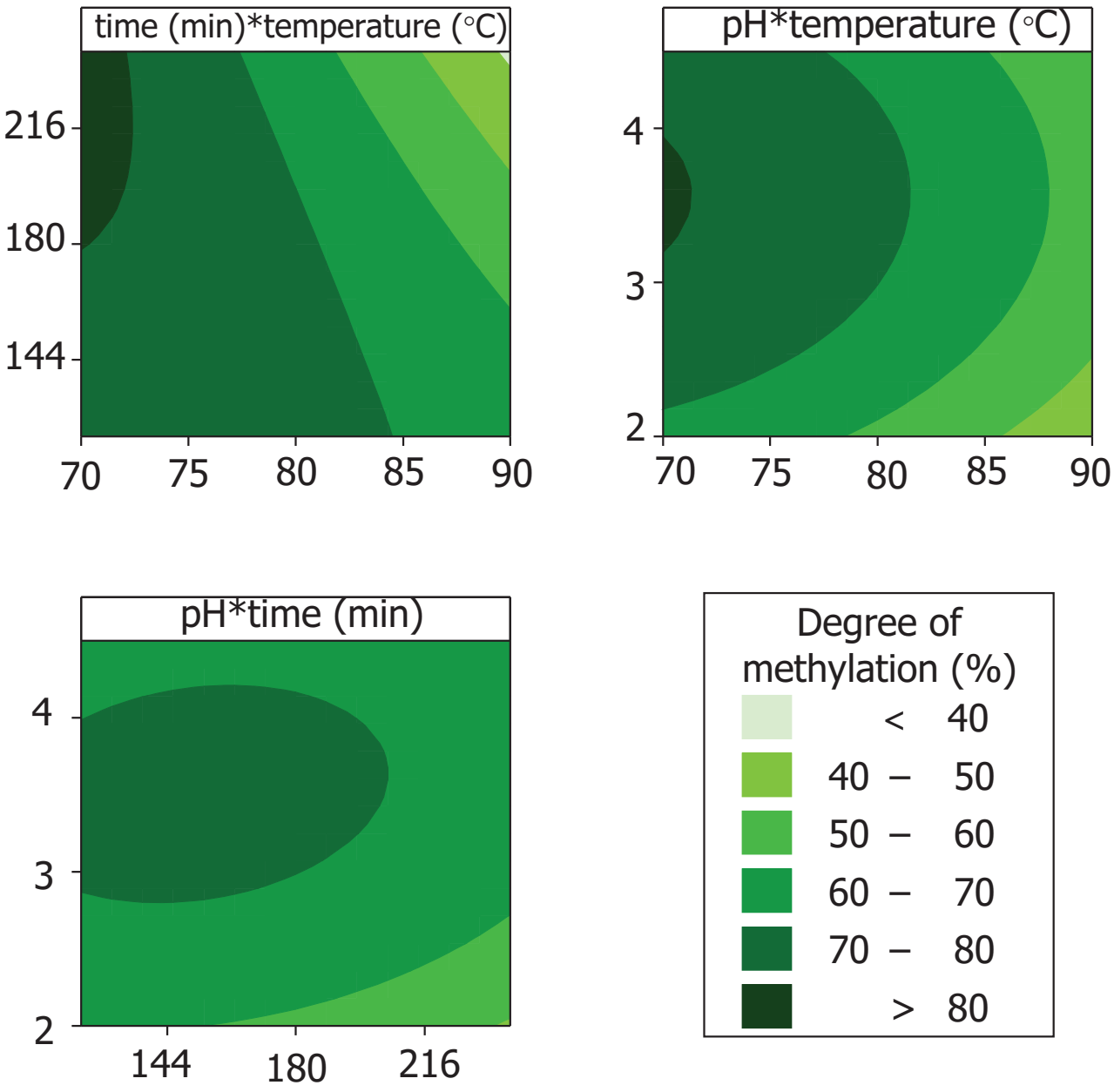

Figure 4 (B&W)

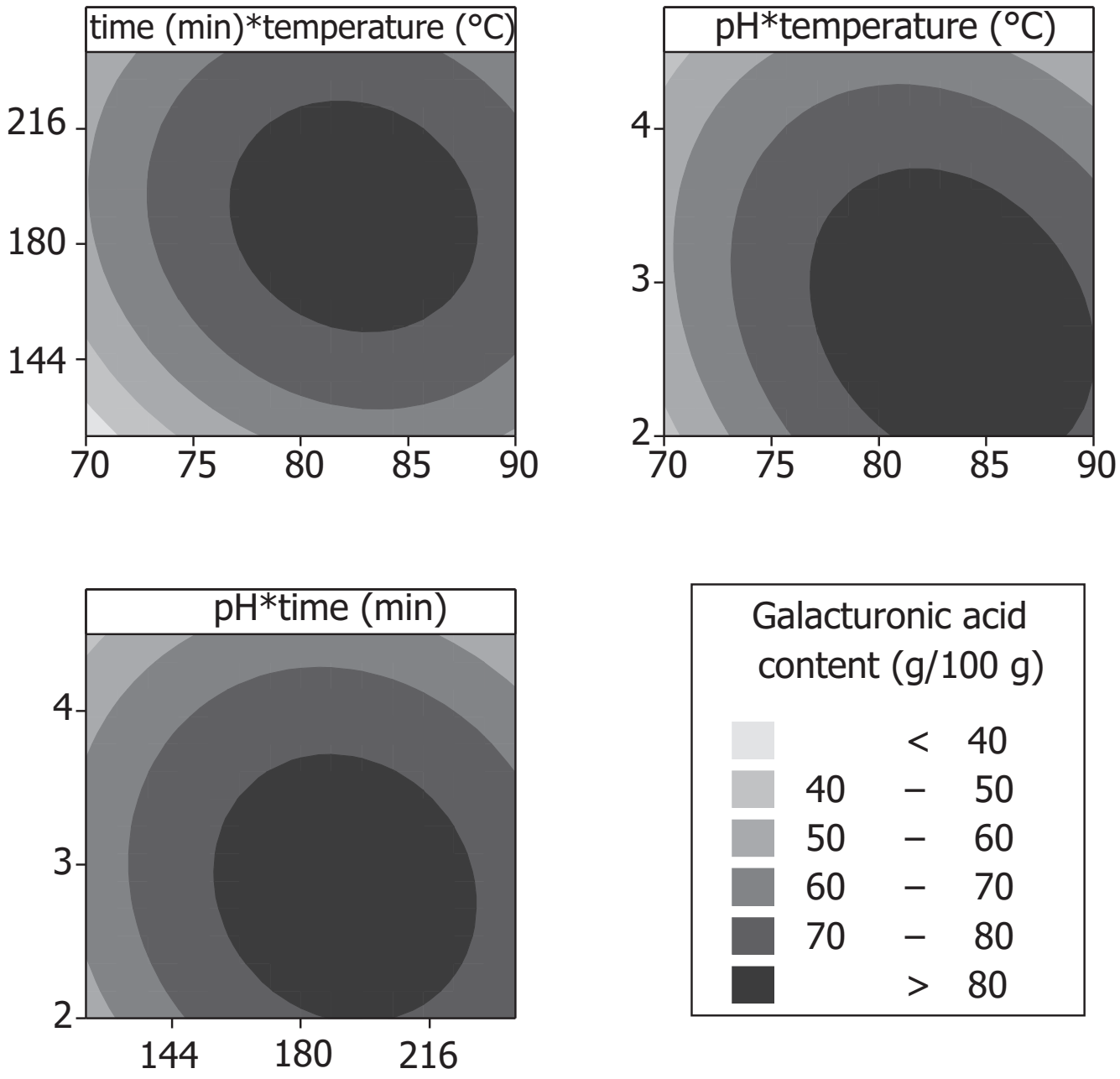
time (min)*temperature (°C)
216
180
144
70
75
80
85
90
pH*temperature (°C)
4
3
2
70
75
80
85
90
pH*time (min)
4
3
2
144
180
216
Galacturonic acid content (g/100 g)
< 40
40 – 50
50 – 60
60 – 70
70 – 80
> 80

Figure 4 (colors)

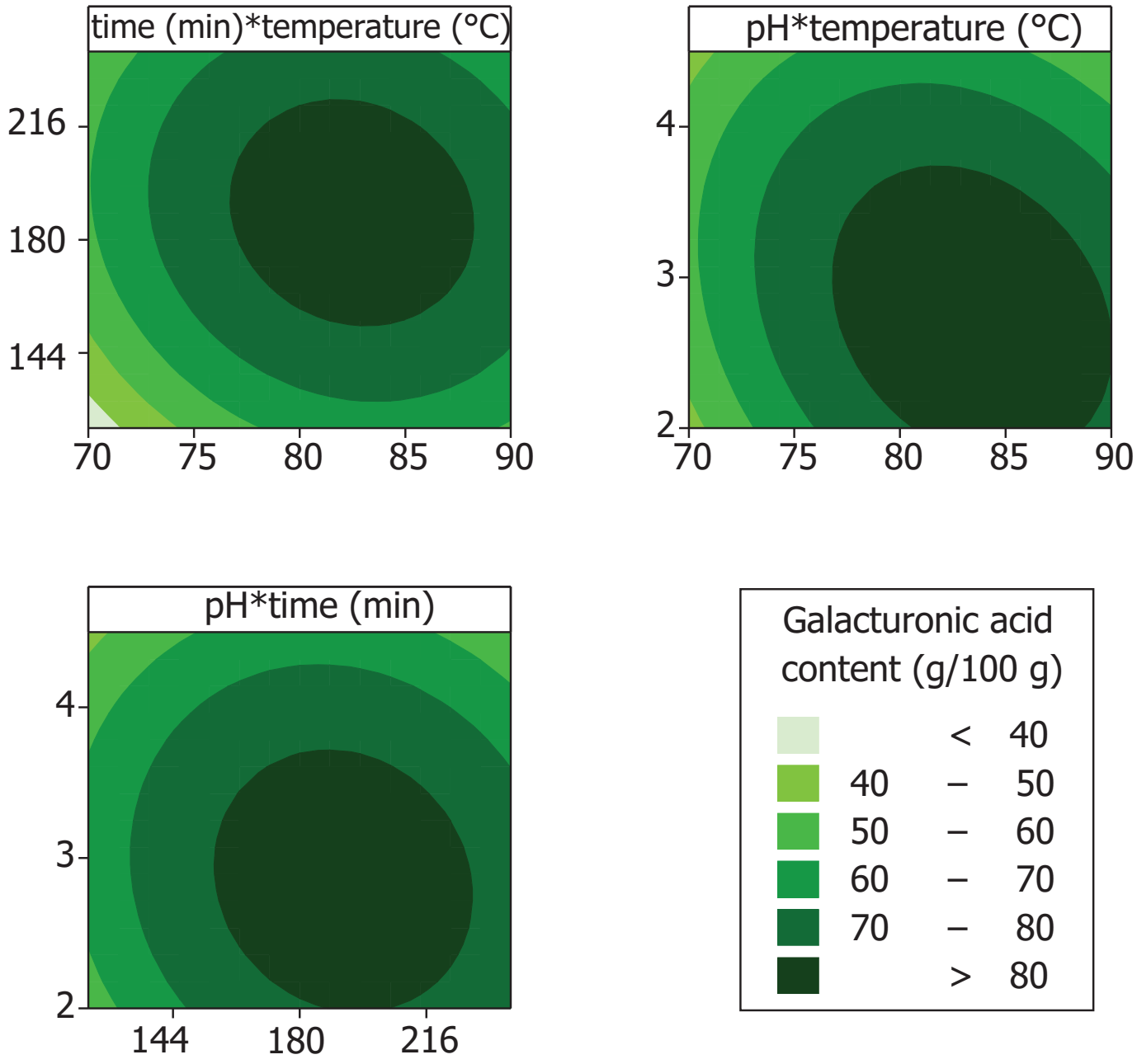

Figure 5 (B&W)

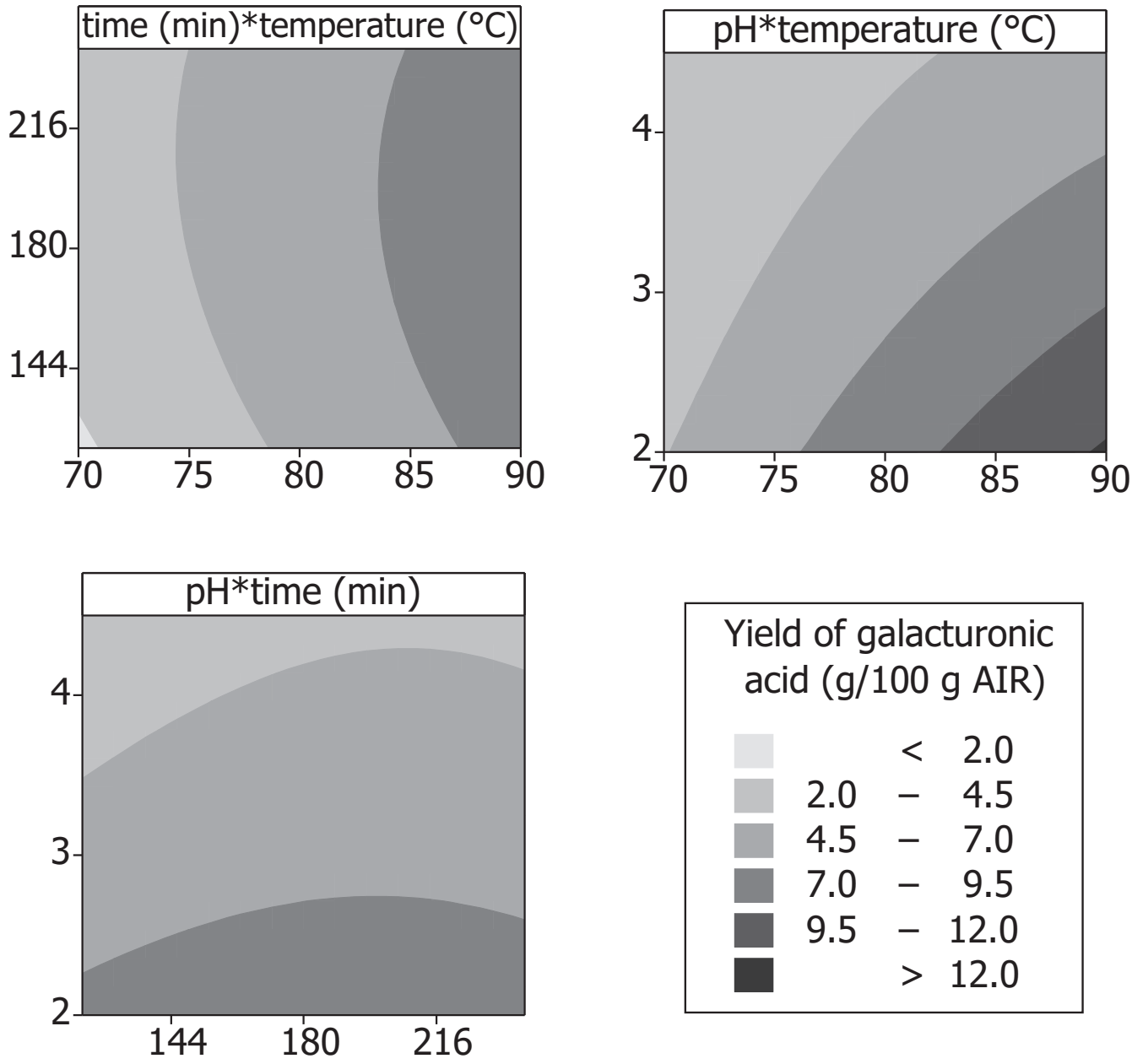
time (min)*temperature (°C)
216
180
144
70
75
80
85
90
pH*temperature (°C)
4
3
2
70
75
80
85
90
pH*time (min)
4
3
2
144
180
216
Yield of galacturonic acid (g/100 g AIR)
< 2.0
2.0 – 4.5
4.5 – 7.0
7.0 – 9.5
9.5 – 12.0
> 12.0

Figure 5 (colors)