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Supplementary Information

Extraction of Pectin in Seriguela (S*pondias Purpurea L.*) Using Experiments Designs: a Tutorial Using the Free Software ''R''

Extração de Pectina em Seriguela (*Spondias purpurea* L.) Usando Planejamentos Experimentais: um Tutorial Utilizando o Software Gratuito ''R''

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1. Rstudio installation guide

RStudio is an integrated development environment for R, this is a free programming environment for data manipulation, and mathematical and statistical calculations, with well-developed graphical visualization. It has several standard packages (also called libraries) already installed, but non-standard packages can be easily installed, just needing an internet connection. Each package has a ready-to-use collection of functions. However, you can write your functions yourself.

2. Download of R

To install R on the Windows® system (version from 3.0.1), just connect to the website (https://cran.r-project.org/bin/windows/base/old/) which will be directed to the download page (Figure 1S). If you want to install it on macOS, just connect to the link (https://cran.r-project.org/bin/macosx/) and download the latest version or the version you want. As shown in Figure 1S, Choose version "R 4.1.1" for Windows® and a new page will appear (Figure 2S). Then download the R 4.1.1 executable file and save it in a folder in your directory.

Figure 1S. Selecting the R version and downloading the executable file.

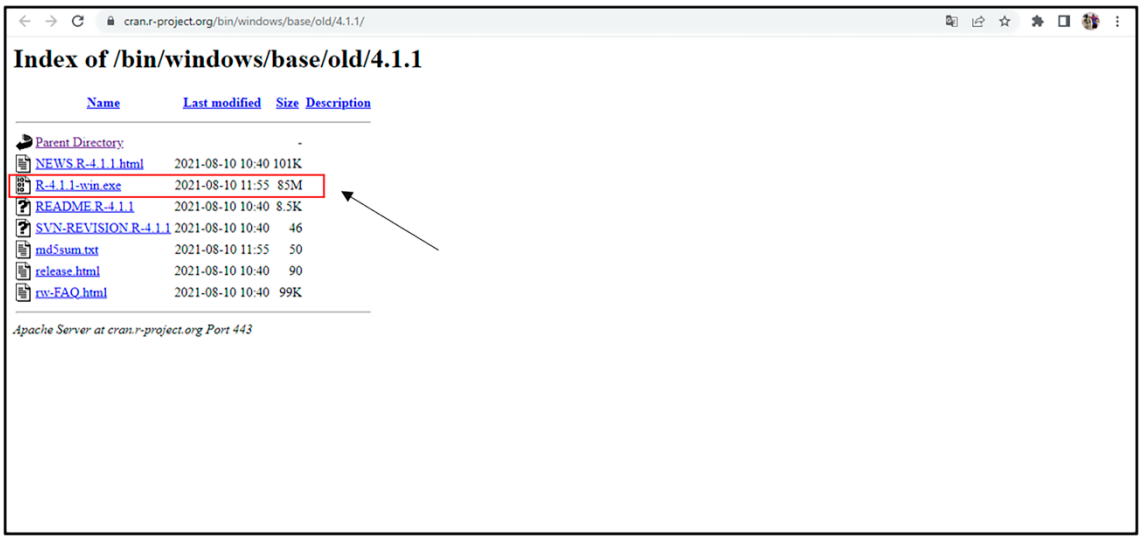


Figure 2S. Download the executable file.

3. Installing R for Windows

After Downloading the executable file, select it with a double click with the left mouse button. Then, a screen will appear as in Figure 3S. Click "Yes". It is noteworthy that this step can be skipped to the next (Figure 4S) depending on the version and settings of the operating system installed.

Figure 3S. Running the file.

After that, a language selection screen for installation will appear, as shown in Figue 4S.

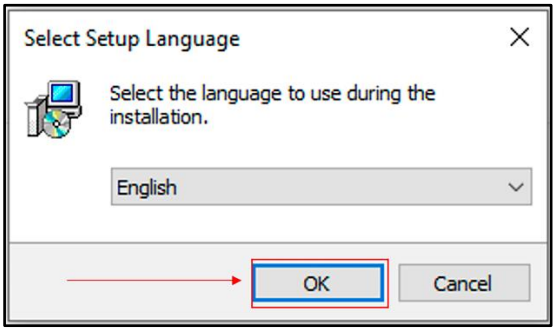


Figure 4S. Language selection.

Then after selecting the language, a screen will appear according to Figure 5S, read the terms and click “next” until the next screen appears.

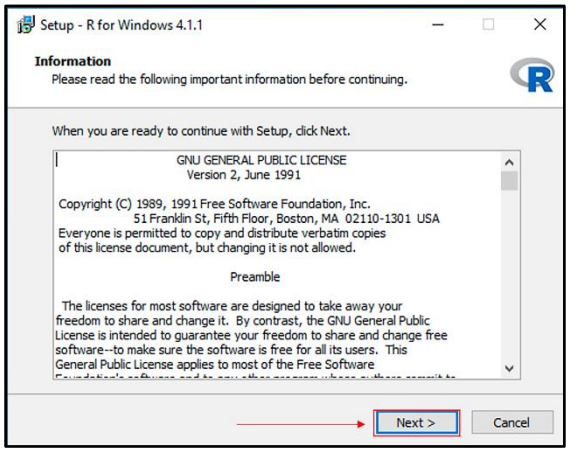


Figure 5S. Term and license.

In Figure 6S, click on “next”, if the installation is done in a different folder, click on “browse” and change the installation directory, then click on “next”, until a new screen appears.

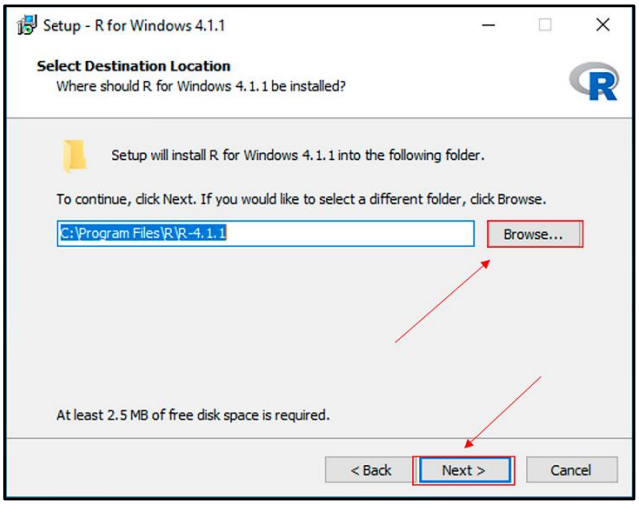


Figure 6S. Selecting the installation target location.

In the frame of selecting components in Figure 7S, click on “next” until the next screen.

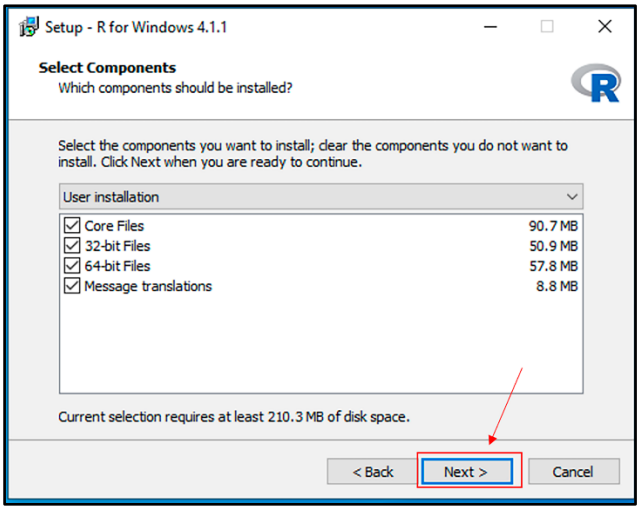


Figure 7S. Selecting installation components.

In Figure 8S, in the boot options, click “No (accept default)” and then “next” until the new screen appears.

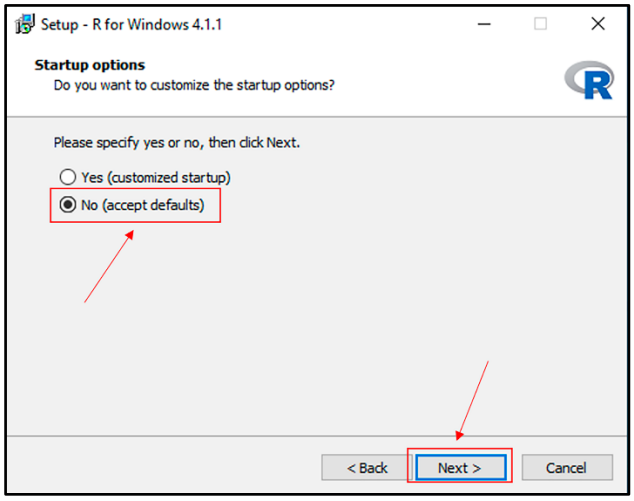


Figure 8S. Boot options.

In Figure 9S, select the folder to install the program shortcuts, and click “next” until a new screen appears.

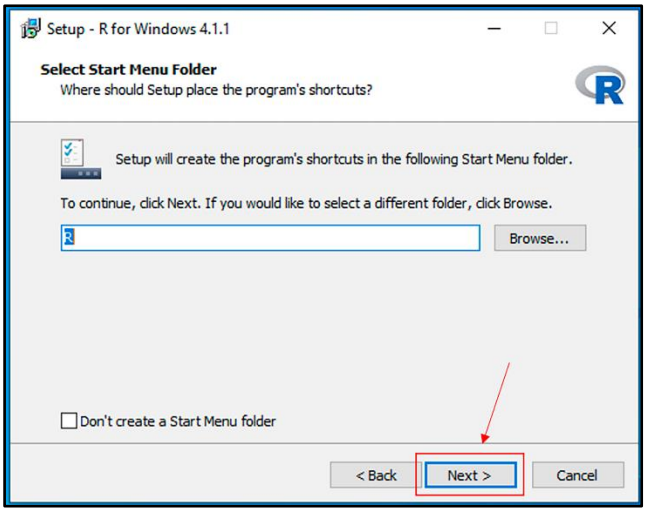


Figure 9S. Select shortcuts folder.

In Figure 10S, on the screen to select additional tasks, click on the options you want to install and on “next”.

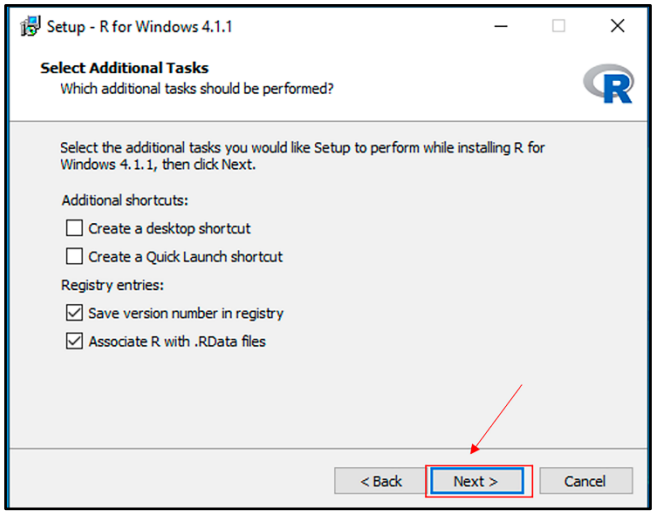


Figure 10S. Select additional tasks.

On the screen represented by Figure 11S(a), follow and wait for the installation of R for Windows on your computer. Upon completion, in Figure 11S(b), click “finish” and it will be ready for use.

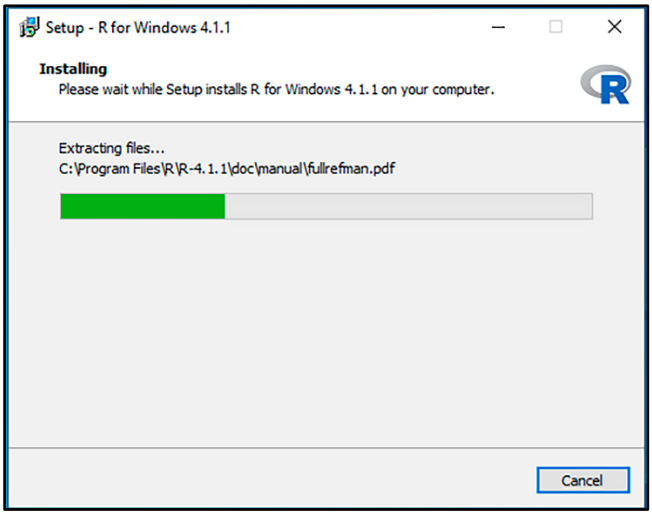


Figure 11S(a). Evolution of the R installation.

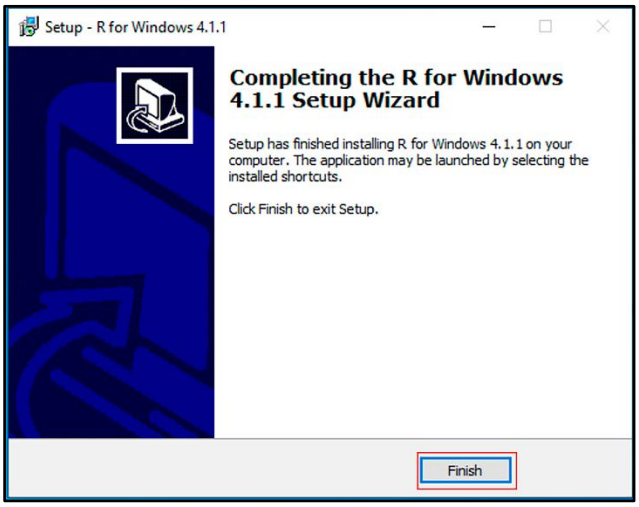


Figure 11S(b). Completion of the installation.

4. Download and install RStudio

4.1. Download

To download RStudio, it is necessary to connect to the website https://www.rstudio.com/products/rstudio/download/ , after which you will be directed to a screen, as shown in Figure 12S, click on the blue bar “Download Rstudio for Windows", then the executable file will be downloaded to the default directory.

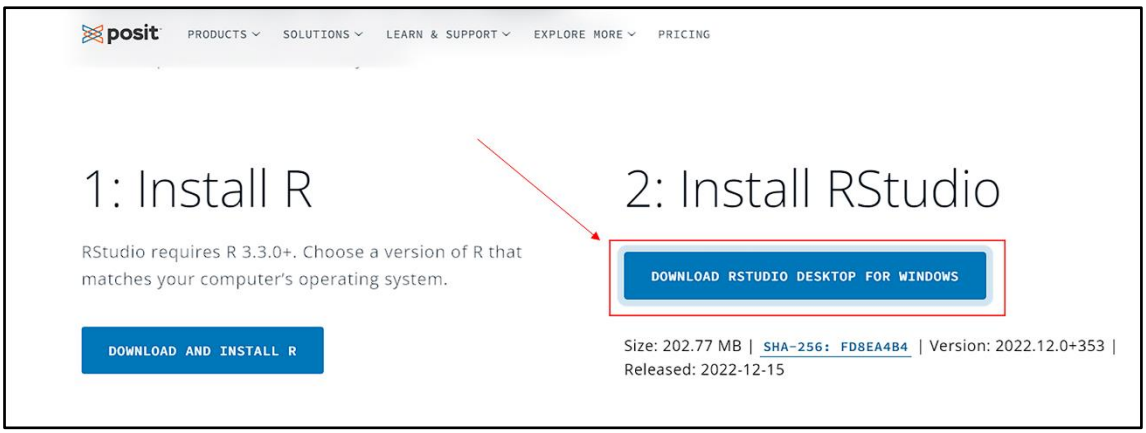


Figure 12S. Downloading the RStudio executable file.

If you want to download and install the latest version of RStudio for macOS, please connect to the link (https://www.rstudio.com/products/rstudio/download/). Also, use the link (https://www.youtube.com/watch?v=rr54DqsJJjY) and watch the video if you have difficulty.

4.2. Installation for Windows

After downloading the executable file, select it in the directory with a double click, a screen will appear as shown in Figure 13S, “Windows permission window”, click on “yes”. After that, a window “Welcome to the RStudio installer” will appear (Figure 14S), click on “next” until the next screen.

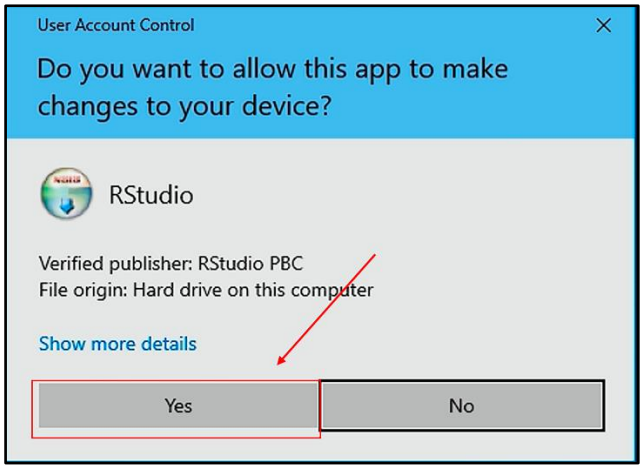


Figure 13S. Permission window.

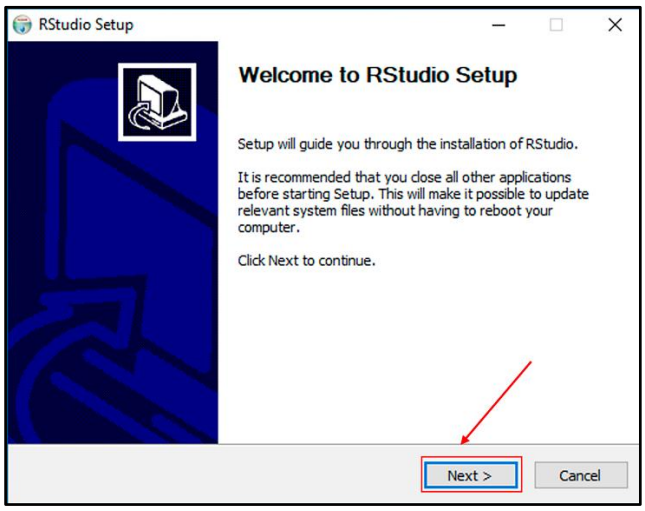


Figure 14S. RStudio Installer

On the screen that will appear, in Figure 15S, choose the destination folder in the installation, and click on “next”. Then a new window (Figure 16S), in it, select the folder in the start menu to save the shortcuts and click on “install”.

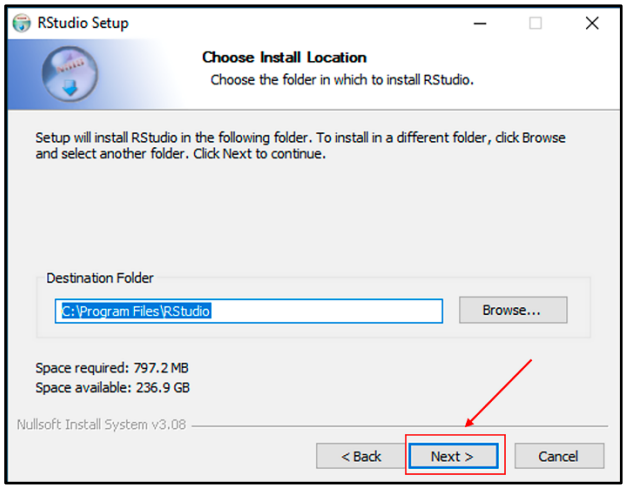


Figure 15S. RStudio installation folder.

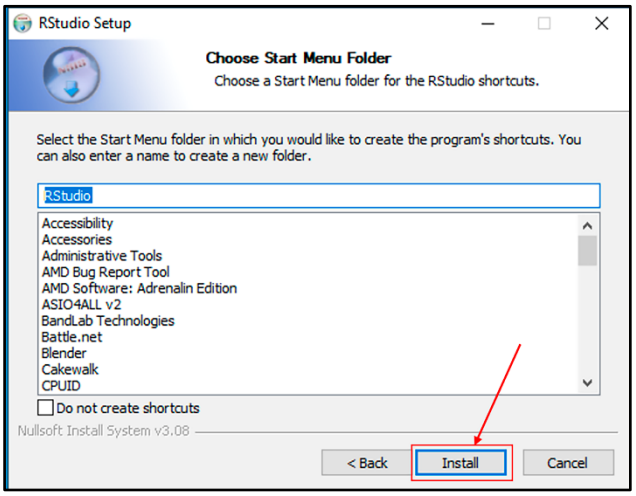


Figure 16S. Saving the shortcuts.

In the window of Figure 17S, wait while your RStudio is installed and click on “finish” on the next screen, Figure 18S. Ready now it will be ready for use.

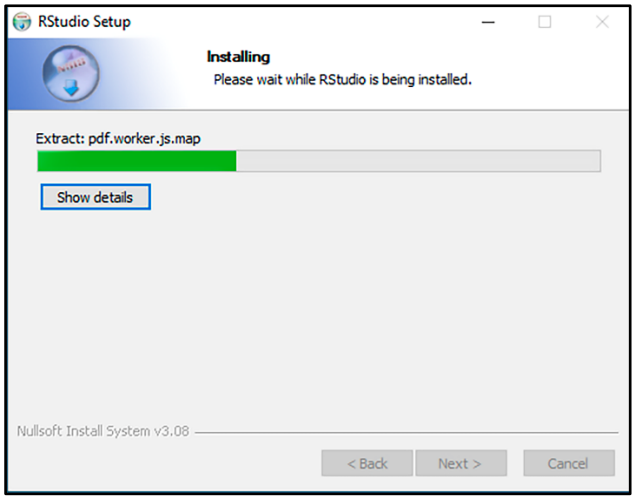


Figure 17S. Evolution of the RStudio installation.

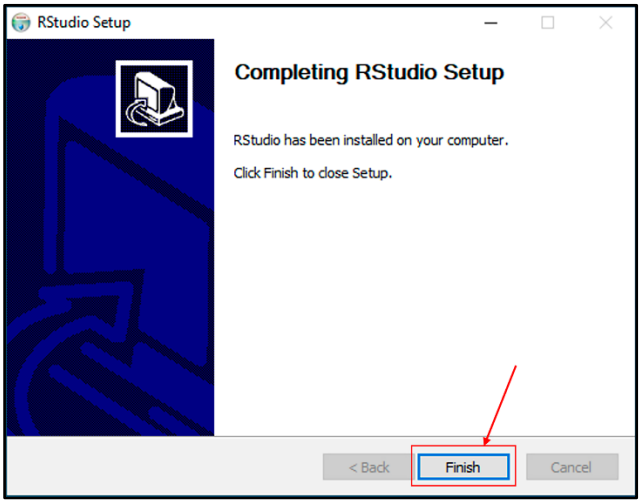


Figure 18S. Completion of the installation.

4.3. RStudio interface

When running the RStudio application, a window will appear to choose the installed R version, 32 or 64 bits (Figure 19S). After choosing, the initial screen with three quadrants will appear (Figure 20S). Colors and fonts may vary depending on your operating system, but the layout will be the same. After creating a new Script with the shortcut keys “Ctrl+Shift+N”, a new window will open with a new layout of the main window, now with four quadrants (Figure 21S).

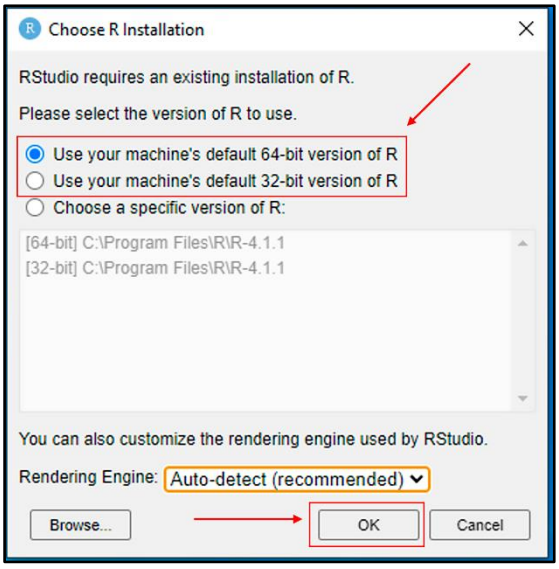


Figure 19S. Selecting the R version.

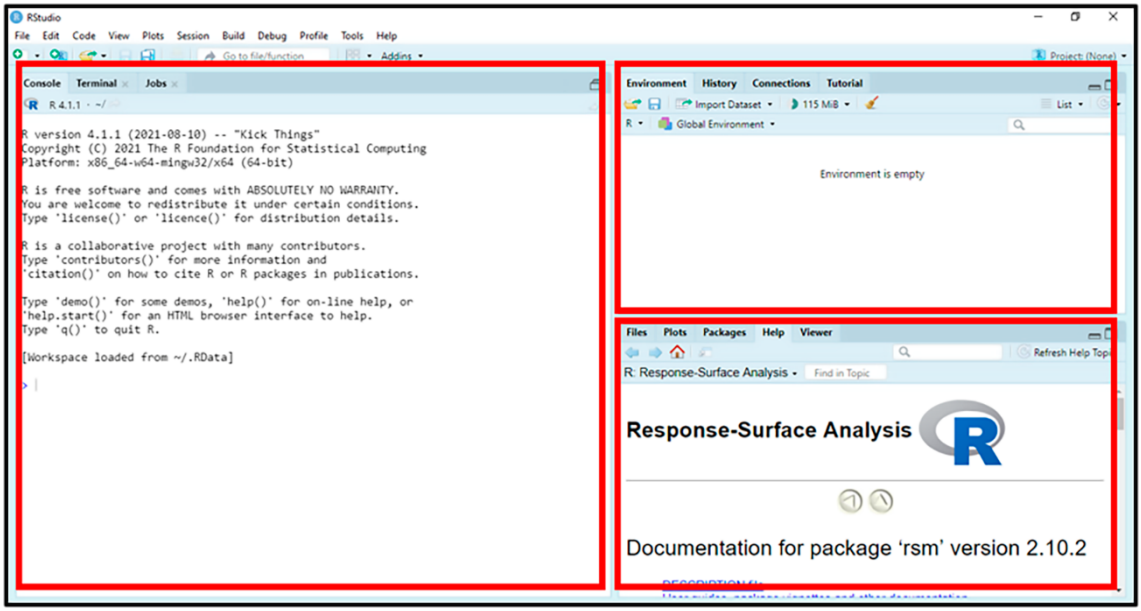


Figure 20S. RStudio main window initial layout.

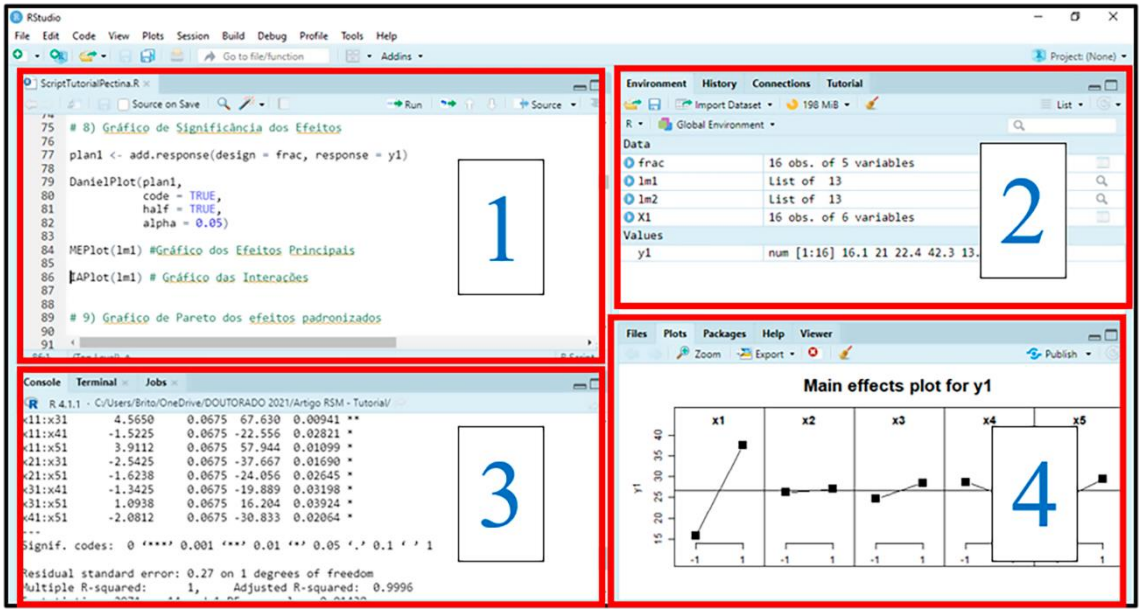


Figure 21S. New layout of RStudio main window after script created.

These quadrants represent the editor (1), the global environment (2), the console (3), and the output (4). The editor and the console are the two main panels in RStudio. They come in this order, but you can organize them any way you like by accessing, in the Tools menu, the Pane Layout section of the Global Options option. Other information:

***Editor/Scripts*:** this is where we write our code. Note that RStudio colors some words and symbols to make the code easier to read.

***Console*:** this is where we run the code and receive the voices.

The other panels are auxiliary. Their goal is to facilitate small tasks that are part of both programming and data analysis, such as looking at the documentation of functions, analyzing the objects created in an R session, searching and organizing the files that make up our analysis, storing and analyzing the graphics created and much more.

***Global Environment*:** panel with all objects created in the session.

***History*:** panel with a history of commands run.

***Files*:** Shows the files in the working directory. It is possible to navigate between directories.

***Graphics and images (Plots)*:** panel where the graphics are presented.

Packages: Displays all installed and loaded packages.

Help: window where function documents are presented.

Visualization (Viewer): panel where reports and dashboards are presented.

4.4. Install and load packages in RStudio

One of the most powerful features of the R language is the fact that anyone can create functions and share them with other R users in an organized way. In this way, many functions are immediately available, which greatly facilitates the work of the data analyst – as long as he knows how to find and install these functions.

A Package is a set of functions within R, usually related to a specific theme. In addition, packages also have documentation. This document explains what each package function is for. It explains how to use each function and even provides practical examples of use.

Most functions are distributed in the form of packages ("packages"), which are made up of R code, help files, datasets, and other important elements for the functioning of the functions.

Packages are usually found on special websites called "repositories". The most important R repository is the CRAN – Comprehensive R Archive Network – accessible at https://cran.r-project.org, and has hundreds of packages ready to be added to your R installation.

There are several ways to install packages. In order of frequency, they are:

a) via CRAN (Comprehensive R Archive Network): by Editor (Figure 21S) install.packages("package-name", dependencies = TRUE) and via the installation button (packages no output) on the RStudio main window (Figure 21S).

b) via Github: devtools::install_github("repo-name/package-name").

c) via file.zip/.tar.gz: install.packages ("C:/path/package-name.zip", repos = NULL)

In this work, the "rsm" package designed to provide R support for response surface methodology will be used. Functions are provided to generate Central Composite and Box-Behnken designs. For the analysis of the resulting contour data, the package allows estimating the response surface, testing its lack of fit, and displaying a set of graphs of the surface. It also implements an encoded data structure to assist in this essential aspect of the methodology.

4.5. Using the install button

Click on the "Packages" quick access tab in the output, (Figure 22S) and then on "Install". A screen will open for you to choose the package you want to install.

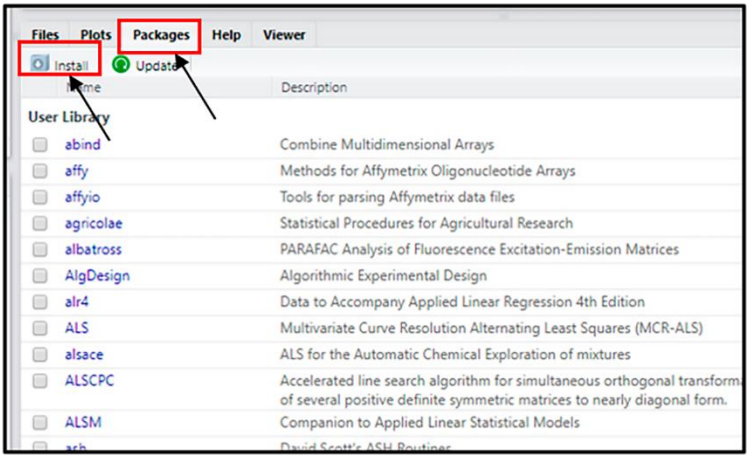


Figure 22S. Quick access tab in the Output window.

In Figure 23S, in the “install packages” window, write the package name “rsm” in “packages” and select where you want to install it in “install from”, that is, from the CRAN repository or a file with an extension “.zip” or “.tar.gz”, click “install” and wait for the installation progress.

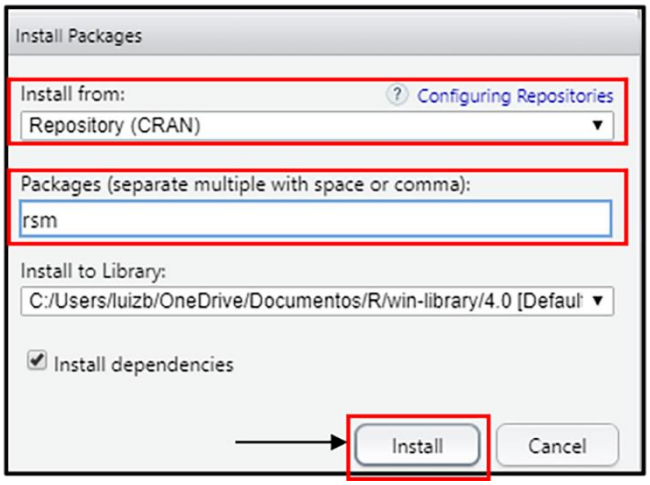


Figure 23S. Installing packages from CRAN or directory.

4.6. Using the install command

Another way to install is using the install command. The first step is to write the command install.packages("rsm", dependencies = TRUE) in the editor window (execute with “ctrl + enter”) or in the console (execute with the “enter” key) as shown in Figure 24S and Figure 25S respectively . Installation progress is followed from the console as shown in Figure 26S.

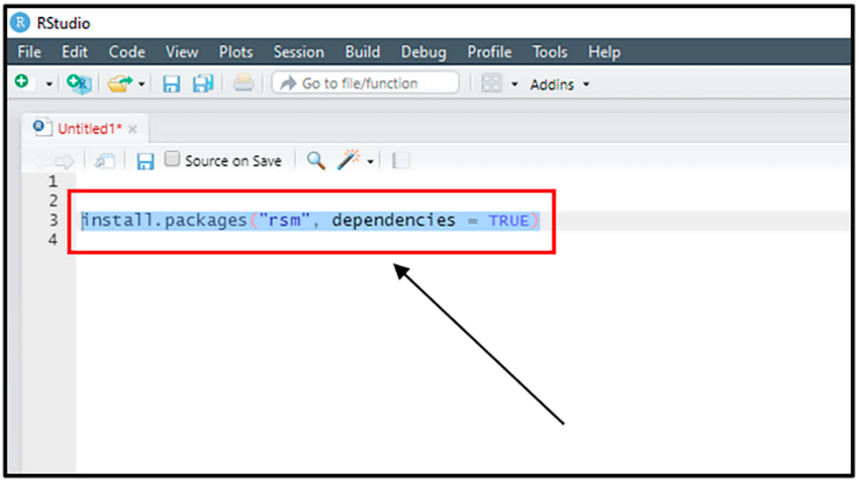


Figure 24S. Installation from the Editor window.

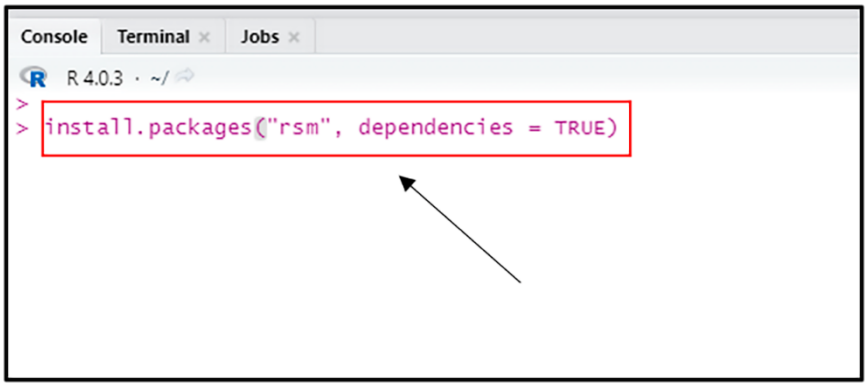


Figure 25S. Direct installation from the Console.

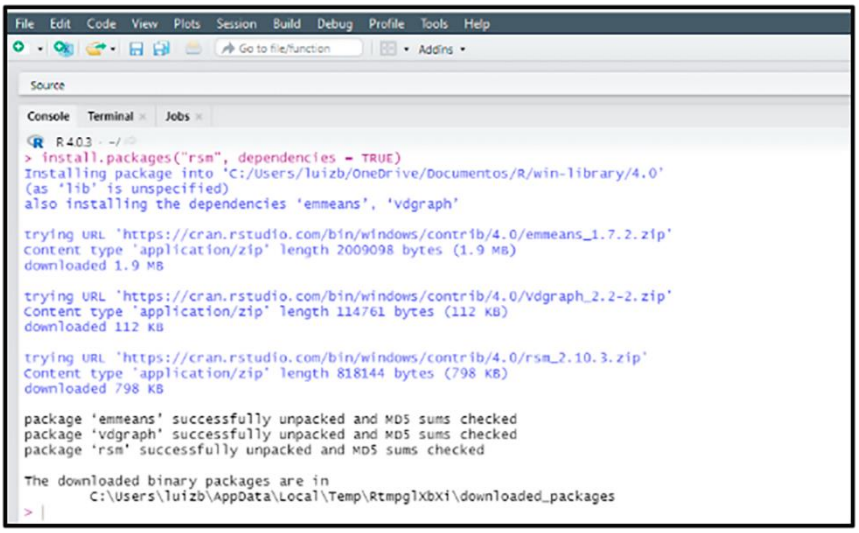


Figure 26S. Progress and completion of the "rsm" package installation.

5. Using the “devtools” package

The network where R packages are stored is known as CRAN. In some cases, the package you want to install will not be available on CRAN. There is a very simple way to resolve this. Several package developers store them on Github.

Imagine that you are looking on Google how to solve a problem and you find a package that will solve your question, but it is not available on CRAN. The package is probably available on Github and can be installed using the Devtools package.

The first step is to install Devtools using the steps in the previous item (which is available to be installed normally over the R network). So would write:

```
install.packages("devtools", dependencies = TRUE)
```

After the “devtools” package is installed, it is necessary to call it to use it using the “library(package name)” command, in which case it would be:

```
library(devtools)
```

The following script must be adapted to the package you want to install. You'll find it on the Github page of the package you're going to install. Probably in the Readme section. To exemplify, the rCharts package will be installed, which is not available for installation in the CRAN using the first methods mentioned here.

```
library(devtools)
install_github('ramnathv/rCharts') # ramnathv is the Github user who created the rCharts package
```

Ready. The above command will install the rCharts package which cannot be installed by the first two ways shown in the previous two items. Following the same reasoning as before, you will need to call the package before using it using the command “library(package name)”.

```
library(rCharts)
```

6. Downloading and Installing the “Rtools” Package

The “Rtools” package serves to install necessary dependencies if the user develops and distributes his R packages. It is worth noting that some packages are only loaded in R if they have installed “Rtools”. Furthermore, it is only necessary for computers using the Windows operating system.

To install “Rtools”, first download it from the link - https://cran.r-project.org/bin/windows/Rtools/history.html. The open window (Figure 27S) will present the executable file version of the “Rtools” package compatible with your installed R. Choose the version compatible with your Windows (32 or 64 bits) and download.

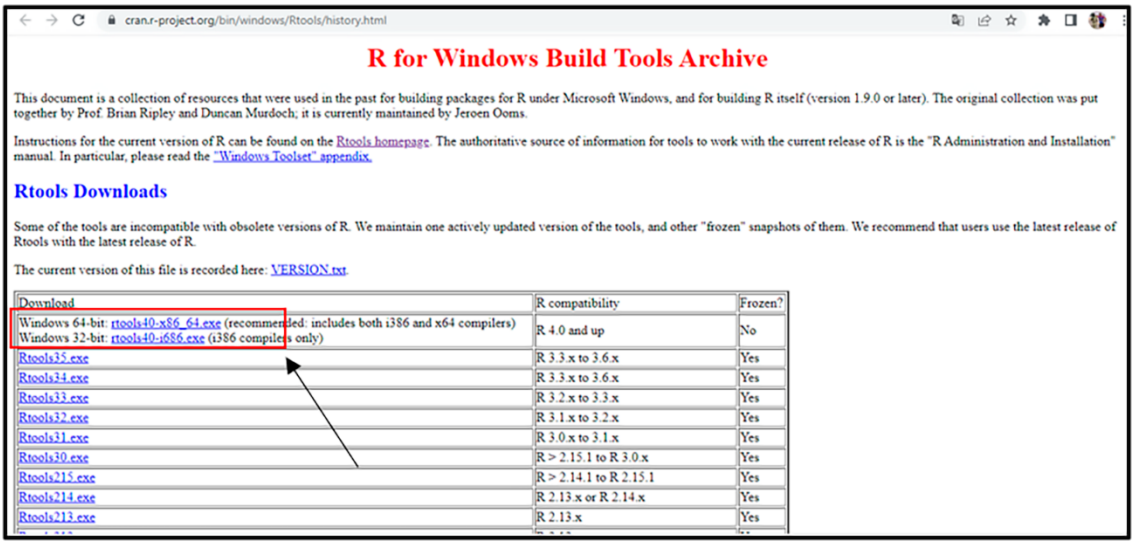


Figure 27S. Download the “Rtools” package.

After Downloading the executable file, select it in the saved directory and with a double click with the left mouse button, a screen will appear as shown in Figure 28S. Then click on “Yes” and a new window will appear. This new window handles user account control.

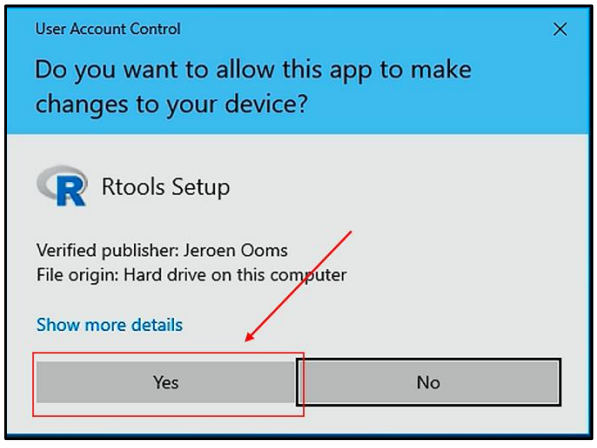


Figure 28S. Running the installer file.

It is noteworthy that this step can be skipped to the next (Figure 29S) depending on the version and settings of the installed operating system. In Figure 29S, click on “Next”, otherwise the installation is in a different folder, click on “Browse” and change the installation directory, then click on “Next”, until a new screen appears.

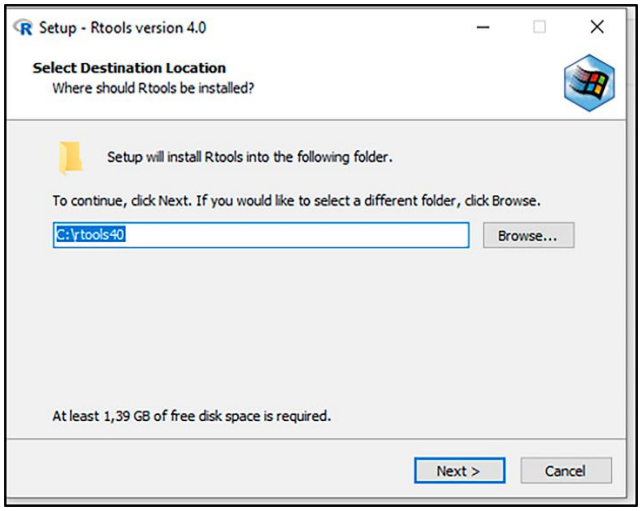


Figure 29S. Selecting the installation location of the “Rtools” package.

On the screen that will appear (Figure 30S) leave only the first option “selected” and click on “Next” and a new window will appear.

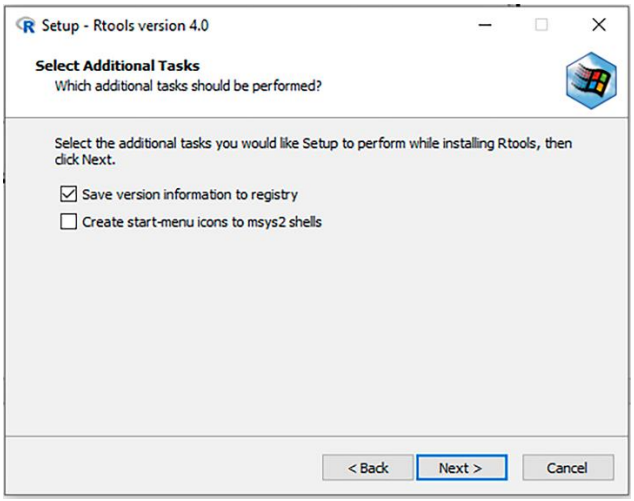


Figure 30S. Selecting additional tasks

Figure 31S shows the Installation Preparation window, click “Install” and follow the installation progress (Figure 32S). When the screen appears as in Figure 33S, the process is complete.

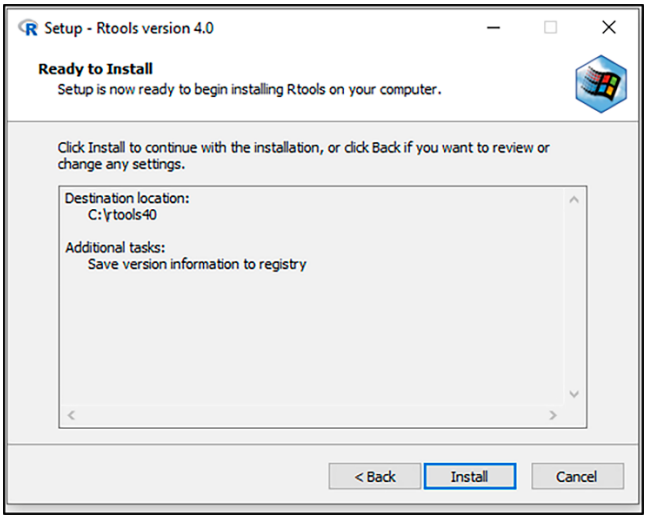


Figure 31S. Preparation window for installing the "Rtools" package.

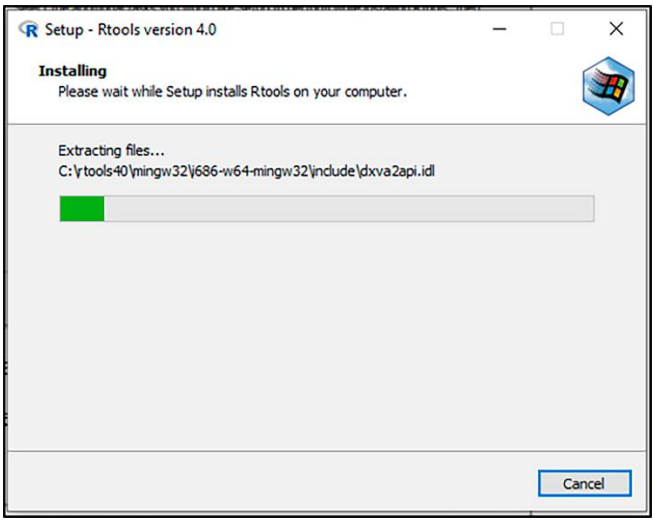


Figure 32S. "Rtools" package installation progress.

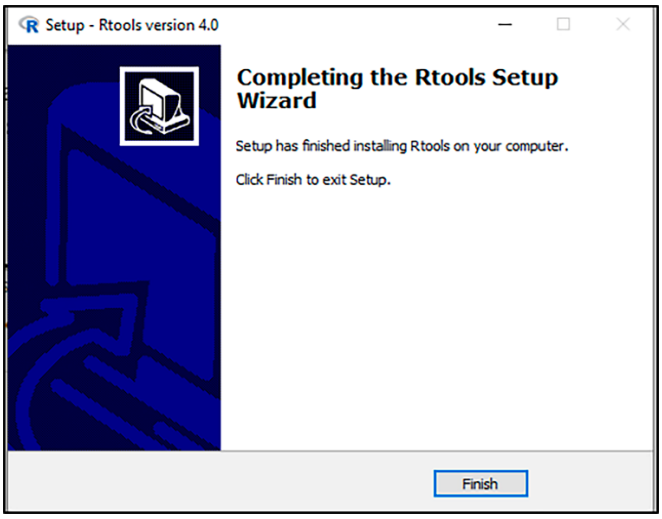


Figure 33S. Completing the installation of the "Rtools" package.

Tables and Figures

Table 1S. Fractional factorial design 2^{5-1} applied in pectin extraction from seriguela peel.

Factors	Units	Coded levels		
		-1	0	1
pH (x_1)	-	1.5	2.1	2.7
Temperature (x_2)	°C	60	70	80
Time (x_3)	min	15	30	45
Power (x_4)	W	1	3	5
Ratio (x_5)	g mL^{-1}	15	22.5	30

Exp.	x_1	x_2	x_3	x_4	x_5	y(%)
1	1.5	60	15	1	30	16.85
2	2.7	60	15	1	15	23.78
3	1.5	80	15	1	15	17.98
4	2.7	80	15	1	30	24.34
5	1.5	60	45	1	15	25.47
6	2.7	60	45	1	30	22.47
7	1.5	80	45	1	30	29.96
8	2.7	80	45	1	15	24.34
9	1.5	60	15	5	15	16.10
10	2.7	60	15	5	30	34.83
11	1.5	80	15	5	30	16.85
12	2.7	80	15	5	15	37.45
13	1.5	60	45	5	30	28.09
14	2.7	60	45	5	15	32.21
15	1.5	80	45	5	15	26.22
16	2.7	80	45	5	30	35.96

17	2.1	70	30	3	22.5	25.43
18	2.1	70	30	3	22.5	25.24
19	2.1	70	30	3	22.5	24.94

Table 2S. Box-Behnken design for optimization of pectin extraction yield.

Factors		Units	Coded levels		
			-1	0	1
pH (x_1)		**-**	2	2.35	2.7
Temperature (x_2)		**°C**	70	75	80
Time (x_3)		**min**	25	35	45
Power (x_4)		**W**	2	3.5	5
Exp.	**x_1**	**x_2**	**x_3**	**x_4**	**y(%)**
1	2	70	35	3.5	18.482
2	2.7	70	35	3.5	17.854
3	2	80	35	3.5	17.472
4	2.7	80	35	3.5	17.417
5	2.35	75	25	2	17.226
6	2.35	75	45	2	18.482
7	2.35	75	25	5	17.636
8	2.35	75	45	5	18.728
9	2	75	35	2	19.274
10	2.7	75	35	2	16.435
11	2	75	35	5	18.100
12	2.7	75	35	5	18.755
13	2.35	70	25	3.5	18.782
14	2.35	80	25	3.5	17.472
15	2.35	70	45	3.5	18.509
16	2.35	80	45	3.5	19.520
17	2	75	25	3.5	16.802
18	2.7	75	25	3.5	17.073
19	2	75	45	3.5	18.022
20	2.7	75	45	3.5	16.965
21	2.35	70	35	2	18.103
22	2.35	80	35	2	18.997
23	2.35	70	35	5	19.729
24	2.35	80	35	5	19.268
25	2.35	75	35	3.5	21.328
26	2.35	75	35	3.5	20.894
27	2.35	75	35	3.5	21.057
28	2.35	75	35	3.5	20.813
29	2.35	75	35	3.5	20.677

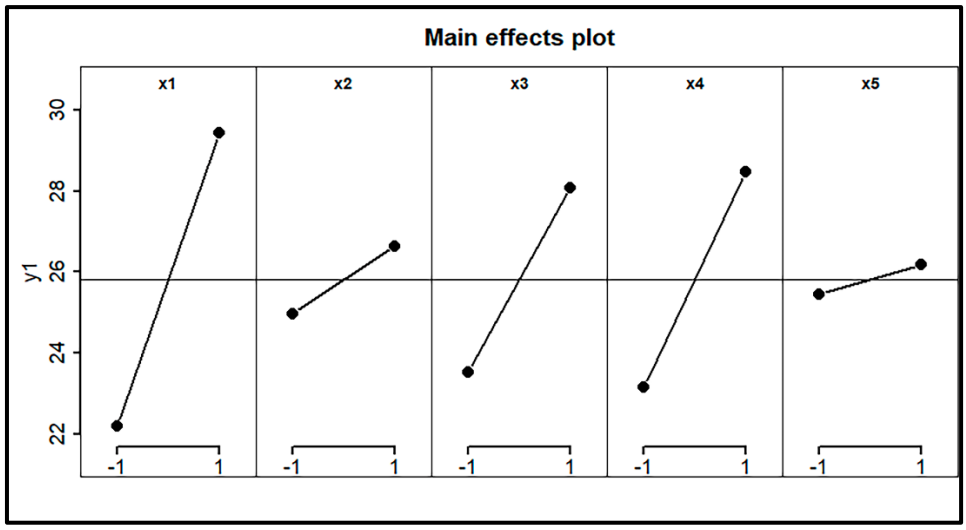


Figure 34S(a). Graphs of main effects.

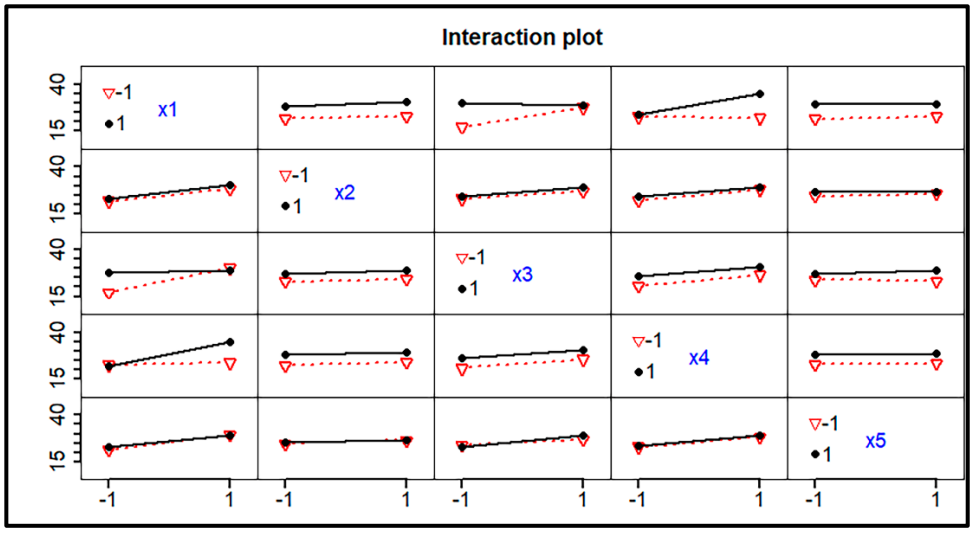


Figure 34S(b). Interactions graph.

```
Call:
FrF2(nruns = 16, ncenter = 3, nfactors = 5, factor.names = c("x1",
    "x2", "x3", "x4", "x5"), randomize = F,
    alias.info = 3)

Experimental design of type  FrF2.center
19  runs

Factor settings (scale ends):
  x1 x2 x3 x4 x5
1 -1 -1 -1 -1 -1
2  1  1  1  1  1

Design generating information:
$legend
[1] A=x1 B=x2 C=x3 D=x4 E=x5

$generators
[1] E=ABCD


Alias structure:
$fi2
 [1] AB=CDE AC=BDE AD=BCE AE=BCD BC=ADE BD=ACE BE=ACD CD=ABE CE=ABD DE=ABC


The design itself:
   x1 x2 x3 x4 x5
1  -1 -1 -1 -1  1
2   1 -1 -1 -1 -1
3  -1  1 -1 -1 -1
4   1  1 -1 -1  1
5  -1 -1  1 -1 -1
6   1 -1  1 -1  1
7  -1  1  1 -1  1
8   1  1  1 -1 -1
9  -1 -1 -1  1 -1
10  1 -1 -1  1  1
11 -1  1 -1  1  1
12  1  1 -1  1 -1
13 -1 -1  1  1  1
14  1 -1  1  1 -1
15 -1  1  1  1 -1
16  1  1  1  1  1
17  0  0  0  0  0
18  0  0  0  0  0
19  0  0  0  0  0
class=design, type= FrF2.center
```

Figure 35S. Summary table of the structure of fractional factorial design 2^{5-1}.

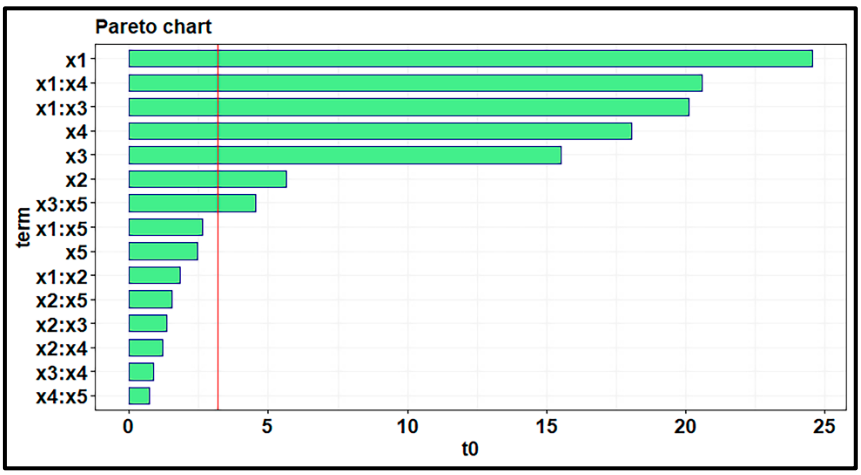


Figure 36S. Pareto chart of standardized effects.

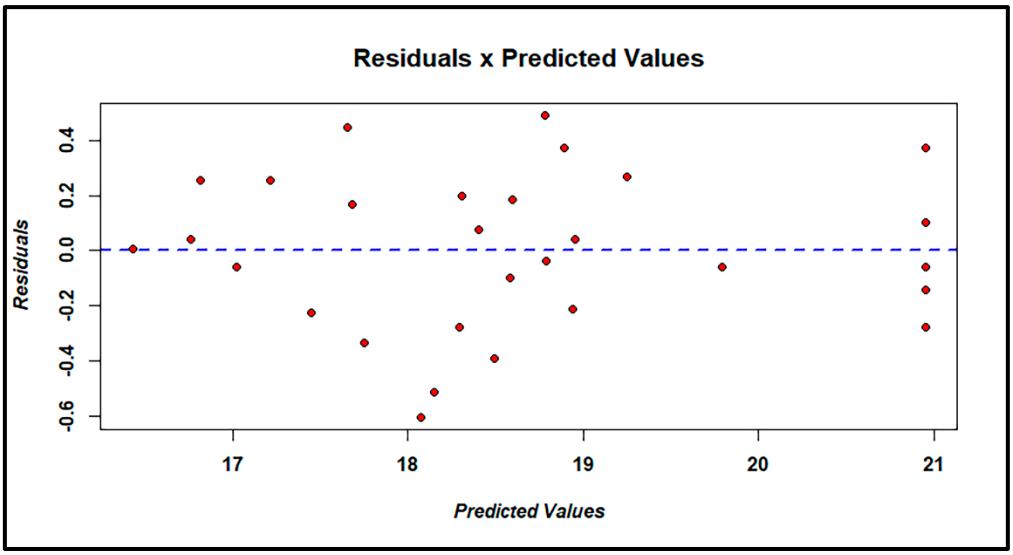


Figure 37S. Graph of the relationship between residual values and experimental values for optimizing pectin extraction.

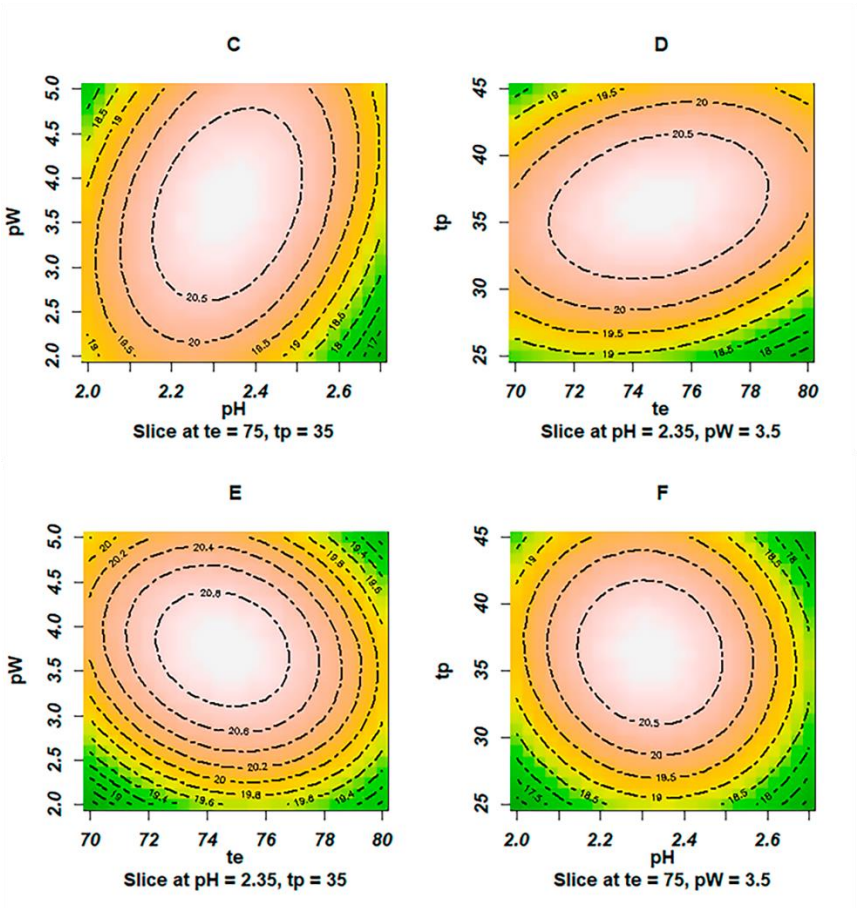


Figure 38S. Contour charts C, D, E and F.

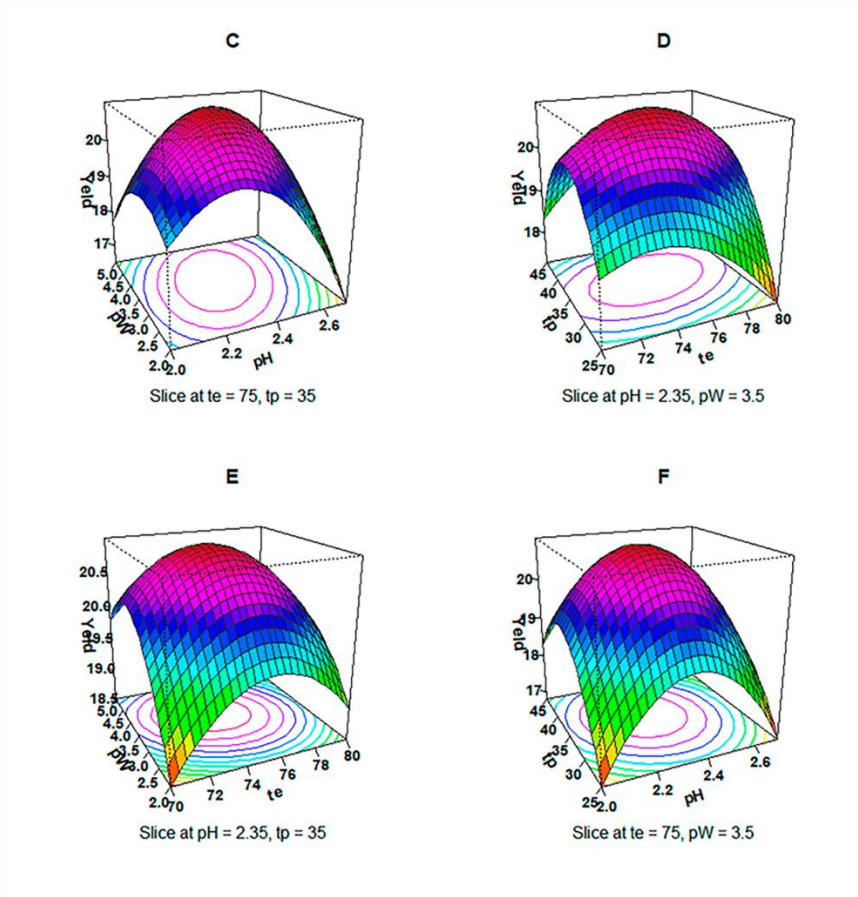


Figure 39S. Response Surface Graphs C, D, E and F.

7. Stationary point and optimization via steepest ascent (path of maximum inclination)

One option is to use steep climb optimization charts. For the construction and formatting of these graphs, type and then press “Ctrl+Enter” in the functions editor the following arguments:

```
otim1 <- steepest(rsm1, dist = seq(0, 1, by = .1), descent = F)
otim1
```

Note that the “otim1” object created will appear in the global environment window and be viewed directly on the console, as shown in Figure 40S.

	dist	x1	x2	x3	x4	pH	te	tp	pw	yhat
1	0.1	-0.041	-0.020	0.069	0.056	2.33565	74.900	35.69	3.5840	20.999
2	0.2	-0.059	-0.052	0.125	0.135	2.32935	74.740	36.25	3.7025	21.013
3	0.3	-0.050	-0.108	0.144	0.235	2.33250	74.460	36.44	3.8525	21.000
4	0.4	-0.029	-0.180	0.136	0.329	2.33985	74.100	36.36	3.9935	20.968
5	0.5	-0.007	-0.255	0.118	0.413	2.34755	73.725	36.18	4.1195	20.921
6	0.6	0.016	-0.330	0.097	0.491	2.35560	73.350	35.97	4.2365	20.859
7	0.7	0.037	-0.405	0.075	0.565	2.36295	72.975	35.75	4.3475	20.782
8	0.8	0.058	-0.479	0.051	0.636	2.37030	72.605	35.51	4.4540	20.692
9	0.9	0.079	-0.553	0.028	0.706	2.37765	72.235	35.28	4.5590	20.588
10	1.0	0.100	-0.626	0.004	0.774	2.38500	71.870	35.04	4.6610	20.470

Figure 40S. Table of the values of the coded and real factors as a function of the distance to the maximum inclination.

Given the table shown in Figure 40S, the encoded values of x_1, x_2, x_3 and x_4 in line 2 are very close to the values presented in the canonical analysis at the stationary point. Then, from these values, the value of the maximum yield of the extraction is found. To do this, type the following commands in the function editor:

```
max <- data.frame(x1 = -0.059, x2 = -0.052, x3 = 0.125, x4 = 0.135)
ymax <- predict(rsm1,max)
ymax
```

Note that the “ymax” object created will appear in the global environment window and the result (21.013) can be viewed directly in the console by typing “ymax” in the editor and pressing “Ctrl+Enter”.

To build the maximum slope optimization graphs type in the editor and press “Ctrl+Enter”:

```
names(max) <- c("x1", "x2", "x3", "x4")
par3 <- par(mfrow = c(1,2))
```

```
# Figure 41S(A)
contour(rsm1, ~x1 + x2, decode = F,lty = 1, at = x_1,
```

```
      xlabs = c("pH", "Temperature"), lwd = 1, cex.lab = 1.1,
      cex.axis =  1.1)

points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x2 ~ x1, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "A")

# Figure 41S(B)
contour(rsm1, ~x1 + x3, decode = F, lty = 1, at = x_1,
      xlabs = c("pH", "Time"), lwd = 1, cex.lab = 1.1,
      cex.axis = 1.1)

points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x3 ~ x1, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "B")

# Figure 41S(C)
ontour(rsm1, ~x1 + x4, decode = F, lty = 1, at = x_1,
      xlabs = c("pH", "Power"), lwd = 1, cex.lab = 1.1,
      cex.axis = 1.1)
points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x4 ~ x1, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "C")

# Figure 41S(D)
contour(rsm1, ~x2 + x3, decode = F, lty = 1, at = x_1,
      xlabs = c("Temperature", "Time"), lwd = 1, cex.lab = 1.1,
      cex.axis = 1.1)
points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x3 ~ x2, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "D")

# Figure 41S(E)
contour(rsm1, ~x2 + x4, decode = F, lty = 1, at = x_1,
      xlabs = c("Temperature", "Power"), lwd = 1, cex.lab = 1.1,
```

```
      cex.axis = 1.1)
points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x4 ~ x2, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "E")

# Figure 41S(F)
contour(rsm1, ~x3 + x4, decode = F, lty = 1, at = x_1,
      xlabs = c("Time", "Power"), lwd = 1, cex.lab = 1.1,
      cex.axis = 1.1)
points(c(bbd1[,3]),c(bbd1[,4]), col = "blue", pch = 19, cex = 1.3)
points(x4 ~ x3, data = otim1, col = "red", pch = 21, bg = "green", cex = 2)
title(main = "F")
```

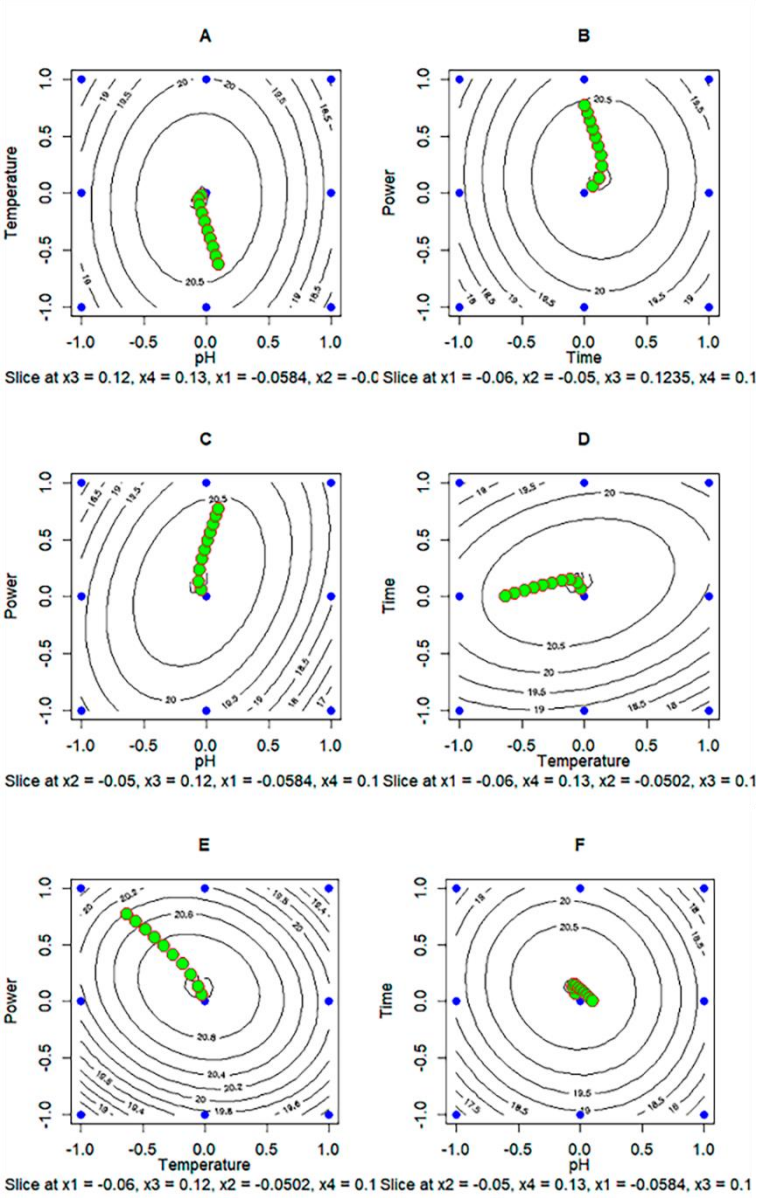


Figure 41S. Maximum Slope Optimization Charts A, B, C, D, E and F.

The Steepest method is a procedure for sequentially moving along this maximum slope - that is, in the direction of maximum response increase (Figure 41S). It is noteworthy that, if minimization is desired, we would call this procedure the method of descent and that for many factors analyzed, canonical analysis is the most recommended.[1]

At this point, although the fitted values are also displayed in Figure 40S (yhat), care must be taken to understand that these are just predictions and that, as the distance increases, they are very bad predictions.[2] What one should do is conduct actual experiments at points along this path and use the observed response values, not these predictions, for guidance on where to locate the next factorial design.[3]

Still in Figure 41S, note that the blue circles correspond to the experimentation region generated by the Box-Benhken design and the green circles represent the path taken from the center to the region closest to the optimum.

8. Comparative study of the results obtained from screening and optimization in Design Expert and Minitab software

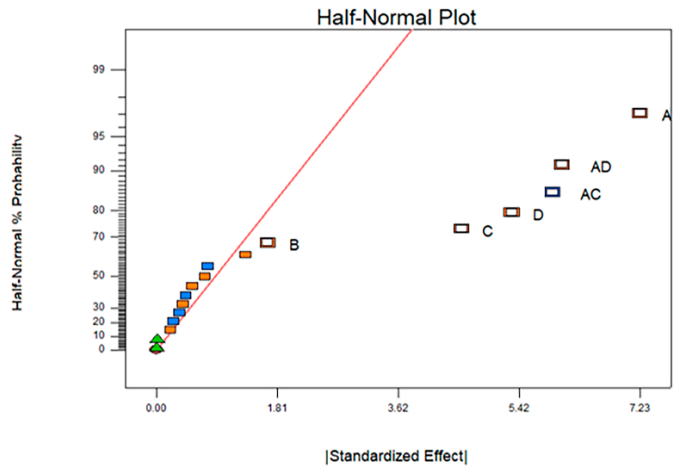


Figure 42S. Normal graph of probability of significance of effects (Design Expert).

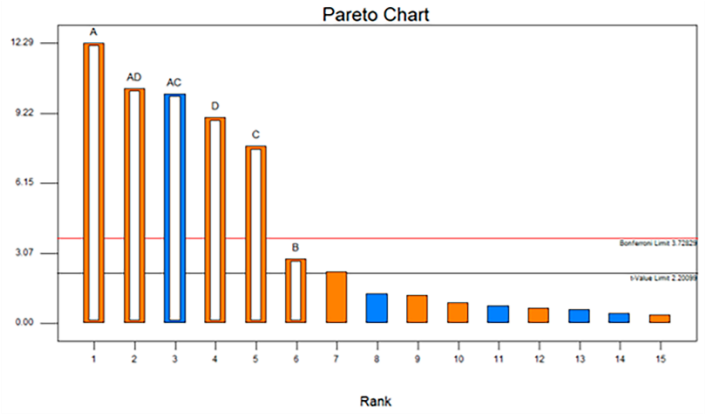


Figure 43S. Pareto Chart of Standardized Effects (Design Expert).

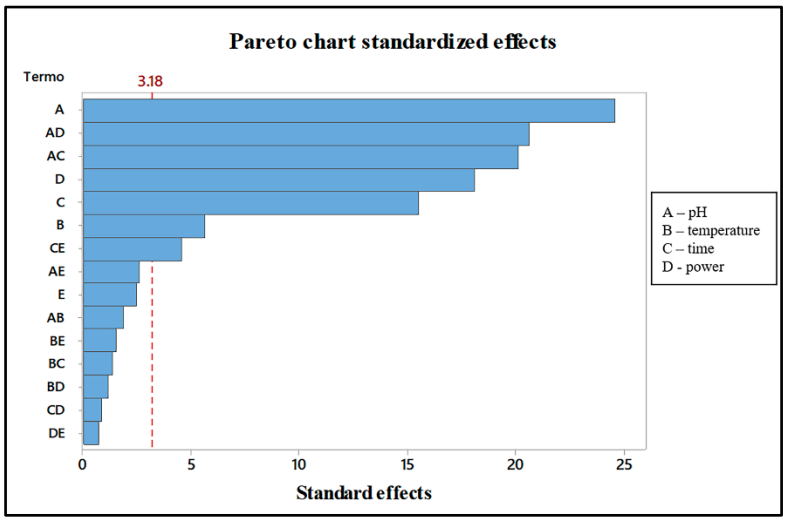


Figure. 44S Pareto chart of standardized effects (Minitab)

Table 3S. Summary table of the significance of the estimated coefficients (Design Expert)

Factor	Coefficients Design Expert
Intercept	25.80625
A-pH	3.61625
B-Temperature	0.83125
C-Time	2.28375
D-Power	2.6575
AC	-2.96125
AD	3.0325
Central point	-0.60292

Table 4S. Anova table (Design Expert).

ANOVA for selected factorial model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	704.1779	6	117.363	84.7356	< 0.0001	significant
A-pH	209.2362	1	209.2362	151.0677	< 0.0001	
B-Temperature	11.05563	1	11.05563	7.982117	0.0165	
C-Time	83.44823	1	83.44823	60.24928	< 0.0001	
D-Power	112.9969	1	112.9969	81.58331	< 0.0001	
AC	140.304	1	140.304	101.2989	< 0.0001	
AD	147.1369	1	147.1369	106.2323	< 0.0001	
Curvature	0.918337	1	0.918337	0.663036	0.4328	not significant
Residual	15.23554	11	1.385049			
Lack of Fit	15.11348	9	1.679275	27.51406	0.0356	significant
Pure Error	0.122067	2	0.061033			
Cor Total	720.3318	18				

The Model's F value of 84.74 implies that the model is significant. The p-values of the terms all less than 0.05 indicating that the terms (A, B, C, D, AC, AD) of the model are significant. The curvature was not significant and there was still a lack of fit. It had an R^2 of 97.88% with a good agreement

between the predicted and the adjusted and still an adequate precision indicating that the model can be used.

It is suggested to analyze the normal probability plot of the student residuals to check the normality of the residuals, student residuals versus predicted values to check for constant error, Externally Studentized residuals to look for outliers, i.e. influential values and the Box-Cox plot for power transformations. If all the model statistics and diagnostic graphs are OK, finish with the Model graphs icon.

The equation gets:

$$Y(\%) = -6.031 + 8.317 * \text{pH} + 0.0831 * \text{Temperature.} + 0.843 * \text{Time} - 3.978 * \text{Power} - 0.329 * \text{pH} * \text{Time} + 2.527 * \text{pH} * \text{Power} \quad \textbf{(1S)}$$

Table 5S. Summary table of the significance of the estimated coefficients (Minitab)

Factor	Coefficients (Design Expert)
Intercept	25.711
A-pH	3.616
B-Temperature	0.831
C-Time	2.284
D-Power	2.657
AC	-2.961
AD	3.033
Central point	-

Table 6S. Anova Table (Minitab)

ANOVA for selected factorial model					
Analysis of variance table [Partial sum of squares - Type III]					
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	719.291	15	47.953	138.27	< 0.001
Linear	418.839	5	83.768	241.54	0,000
A-pH	209.236	1	209.236	603.03	< 0.0000
B-Temperature	11.056	1	11.056	31.08	0,011
C-Time	83.448	1	83.448	240.62	< 0.001
D-Power	112.997	1	112.997	325.83	< 0.0000
AC	140.304	1	140.304	404.57	< 0.0000
AD	147.137	1	147.137	424.27	< 0.0000
Curvature					
Residual	1.040	3	0.347		
Lack of Fit	0.918	1	0.918	27.51406	0.0356
Pure Error	0.122	2	0.061		
Cor Total	720.332	18			

Model Summary

S	**R^2**	**R^2(aj)**	**R^2(pred)**
0.588898	99.86%	99.13%	0.00%

Regression Equation in Uncoded Units

$$\begin{aligned} C11 = {} & 25.711 + 3.616\,X1 + 0.831\,X2 + 2.284\,X3 + 2.657\,X4 + 0.363\,X5 \\ & + 0.269\,X1 * X2 - 2.961\,X1 * X3 + 3.033\,X1 * X4 - 0.385\,X1 * X5 \\ & + 0.199\,X2 * X3 - 0.175\,X2 * X4 - 0.222\,X2 * X5 - 0.128\,X3 * X4 \\ & + 0.668\,X3 * X5 + 0.106\,X4 * X5 \end{aligned} \quad \textbf{(2S)}$$

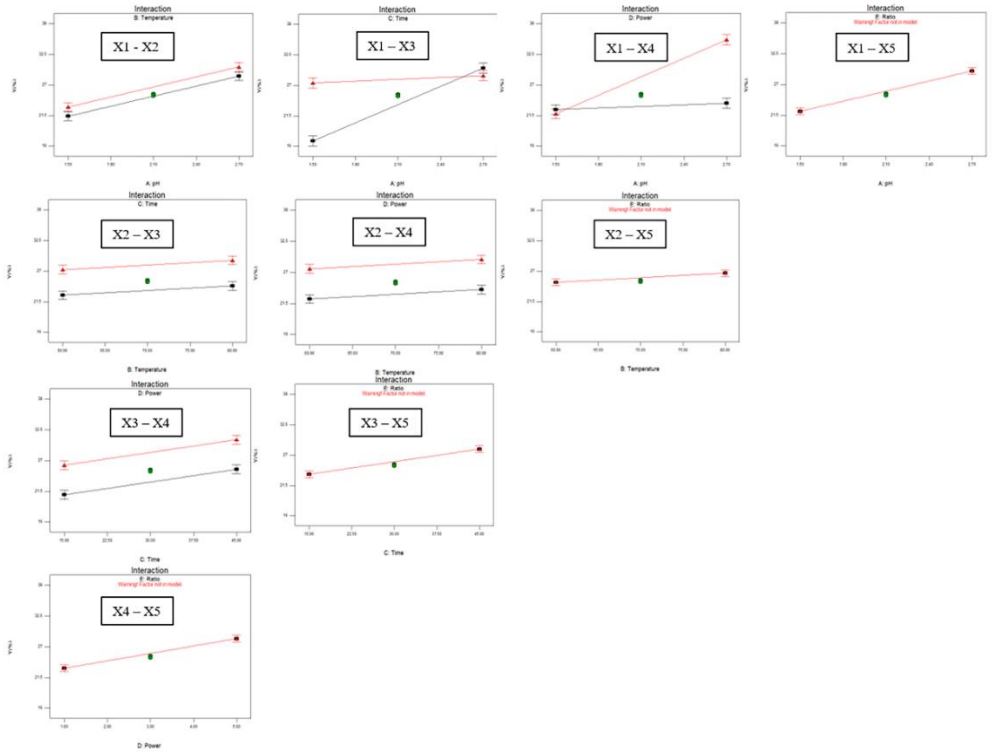


Figure 45S. Main Interactions in the Screening of Variables phase (Design Expert).

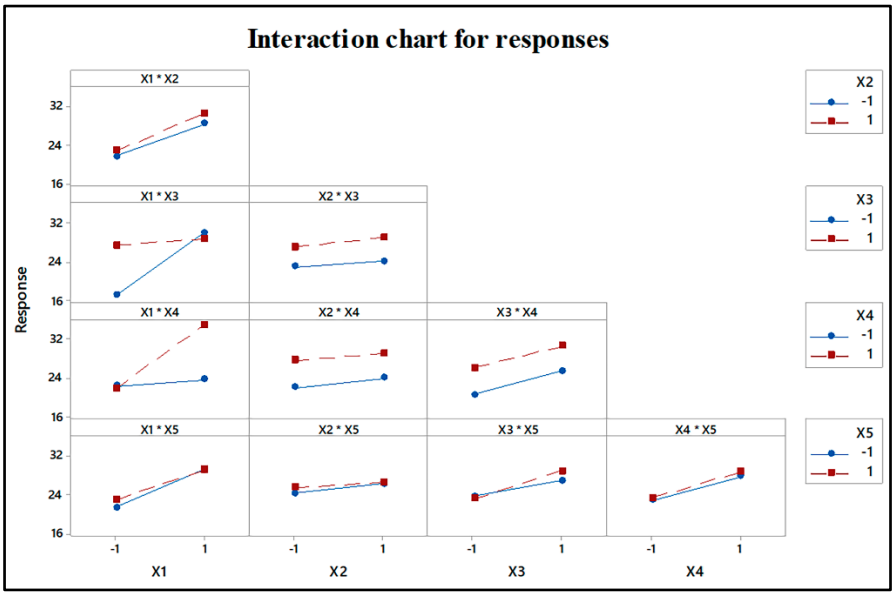


Figure 46S. Main Interactions in the Screening of Variables phase (Minitab).

9. Optimization

9.1. Optimization Design Expert

Table 7S. ANOVA summary table and regression coefficients.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	50.00046	14	3.571461	21.48901	< 0.0001	significant
A-pH	1.112034	1	1.112034	6.690962	0.0215	
B-Temperatura	0.143664	1	0.143664	0.864408	0.3683	
C-Tempo	2.588194	1	2.588194	15.57282	0.0015	
D-Potência	0.94136	1	0.94136	5.664039	0.0321	
AB	0.082082	1	0.082082	0.493878	0.4937	
AC	0.440896	1	0.440896	2.652813	0.1257	
AD	3.052009	1	3.052009	18.36354	0.0008	
BC	1.34676	1	1.34676	8.103279	0.0129	
BD	0.459006	1	0.459006	2.76178	0.1188	
CD	0.063001	1	0.063001	0.379069	0.5480	
A^2	26.96506	1	26.96506	162.2452	< 0.0001	
B^2	5.485986	1	5.485986	33.00845	< 0.0001	
C^2	18.21821	1	18.21821	109.6166	< 0.0001	
D^2	6.311254	1	6.311254	37.97398	< 0.0001	
Residual	2.326792	14	0.166199			
Lack of Fit	2.076097	10	0.20761	3.312549	0.1298	not significant
Pure Error	0.250695	4	0.062674			
Cor Total	52.32725	28				

The model as well as the terms A, C, D, AD, BC, A^2, B^2, C^2, D^2 are significant. The “lack of fit” was not significant relative to the pure error.

9.2. Optimization Minitab

Table 8S. ANOVA summary table and regression coefficients.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	50.0811	14	3.5772	22.49	< 0.000	
A-pH	1.1120	1	1.1120	6.99	0.019	
B-Temperatura	0.1437	1	0.1437	0.90	0.358	
C-Tempo	2.2838	1	2.2838	14.36	0.002	
D-Potência	1.1402	1	1.1402	7.17	0.018	
AB	0.0821	1	0.0821	0.52	0.484	
AC	0.4409	1	0.4409	2.77	0.118	
AD	3.0520	1	3.0520	19.19	0.001	
BC	1.3468	1	1.3468	8.47	0.011	
BD	0.4590	1	0.4590	2.89	0.111	
CD	0.0067	1	0.0067	0.04	0.84	
A^2	26.5938	1	26.5938	167.21	< 0.000	
B^2	5.3192	1	5.3192	33.45	< 0.000	
C^2	18.8357	1	18.8357	118.43	< 0.000	
D^2	6.6768	1	6.6768	41.98	< 0.000	
Residual	2.2266	14	0.1590			
Lack of Fit	1.9759	10	0.1976	3.15	0.140	not significant
Pure Error	0.2507	4	0.0627			
Cor Total	52.3077	28				

Std. Dev.	0.407676	**R-Squared**	0.955534
Mean	18.62793	**Adj R-Squared**	0.911068
C.V. %	2.188518	**Pred R-Squared**	0.763985
PRESS	12.35003	**Adeq Precision**	15.29102

The R^2 was 95.55%. The “Pred R-Squared” of 0.7640 is in reasonable agreement with the “Adj R-Squared” of 0.9111. “Adeq Precision” measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Its ratio of 15.291 indicates an adequate signal. This template can be used to navigate the design space.

Table 9S. Comparative table of coefficients (Design Expert x Minitab).

Factor	Coefficients	
	Design Expert	**Minitab**
Intercept	20.9538	20.954
A-pH	-0.30442	-0.304
B-Temperature	-0.10942	-0.109
C-Time	0.464417	0.436
D-Power	0.280083	0.308
AB	0.14325	0.143
AC	-0.332	-0.332
AD	0.8735	0.873
BC	0.58025	0.580
BD	-0.33875	-0.339
CD	-0.1255	-0.041
A^2	-2.0389	-2.025
B^2	-0.91965	-0.906
C^2	-1.6759	-1.704
D^2	-0.9864	-1.015

Model equation:

Design expert

$$Y\% = -264.486 + 68.715 * \text{pH} + 5.056 * \text{Temperature} + 0.601 * \text{Time} + 3.026 * \text{Power} + 0.0819 * \text{pH} * \text{Temperature} - 0.095 * \text{pH} * \text{Time} + 1.664 * \text{pH} * \text{Power} + 0.012 * \text{Temperature} * \text{Time} - 0.0452 * \text{Temperature} * \text{Power} - 8.367\text{E}-003 * \text{Time} * \text{Power} - 16.644 * \text{pH}^2 - 0.037 * \text{Temperature}^2 - 0.0168 * \text{Time}^2 - 0.438 * \text{Power}^2 \quad \textbf{(3S)}$$

Minitab

$$C9 = 20.954 - 0.304\,\text{X1} - 0.109\,\text{X2} + 0.436\,\text{X3} + 0.308\,\text{X4} - 2.025\,\text{X1} * \text{X1} - 0.906\,\text{X2} * \text{X2} - 1.704\,\text{X3} * \text{X3} - 1.015\,\text{X4} * \text{X4} + 0.143\,\text{X1} * \text{X2} - 0.332\,\text{X1} * \text{X3} + 0.873\,\text{X1} * \text{X4} + 0.580\,\text{X2} * \text{X3} - 0.339\,\text{X2} * \text{X4} - 0.041\,\text{X3} * \text{X4} \quad \textbf{(4S)}$$

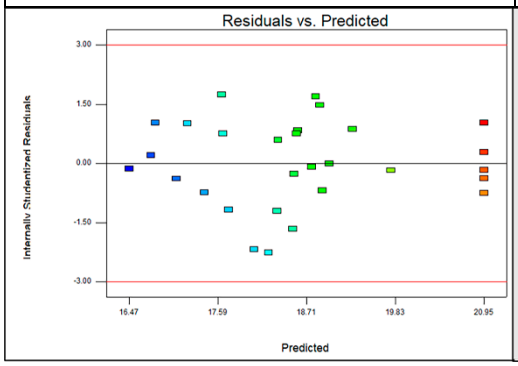
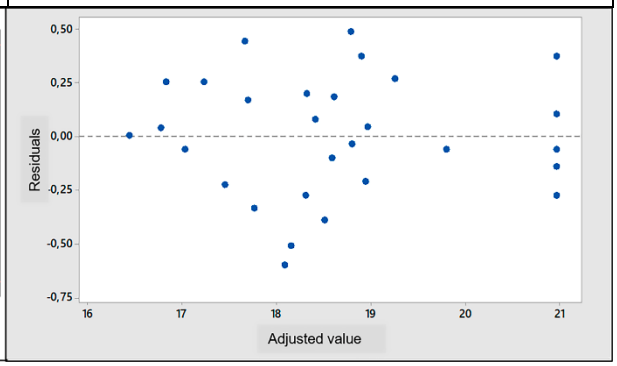
Design expert	Minitab
	

Figure 47S. Graph of the relationship between residual values and experimental values for optimizing pectin extraction (Design Expert x Minitab).

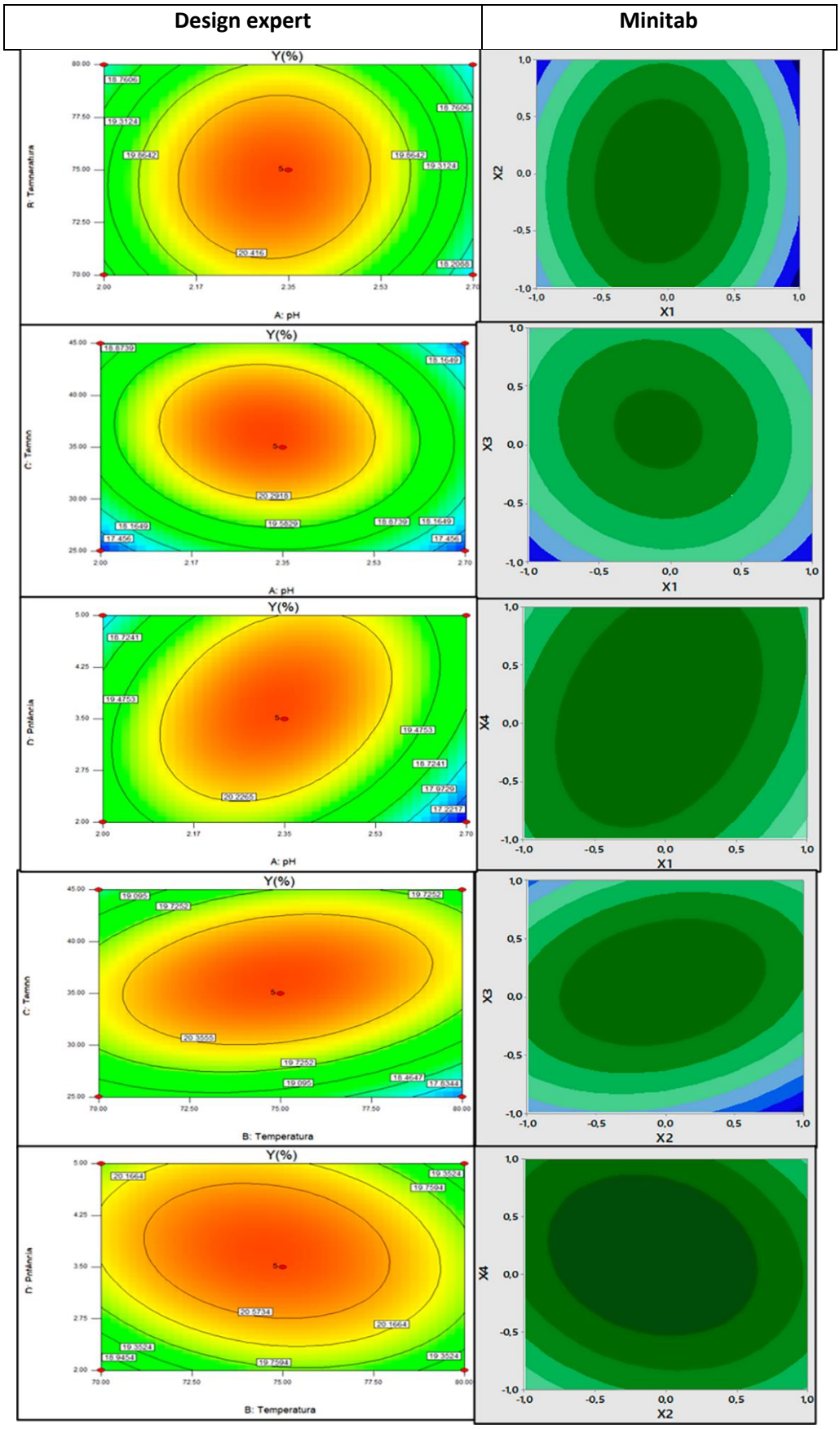


Figure 48S. Contour Charts (Design Expert x Minitab).

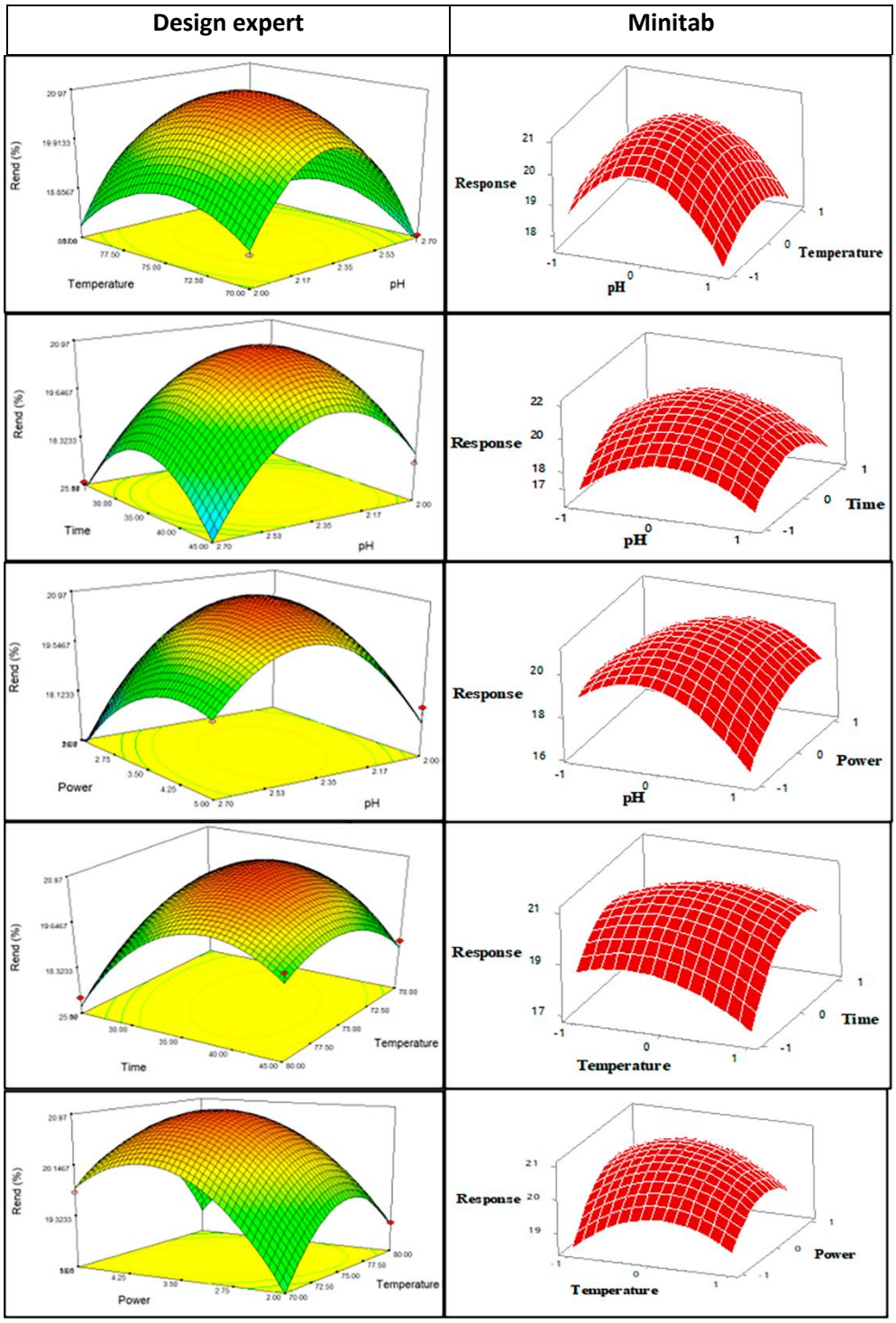


Figure 49S. Response Surface Graphs (Design Expert x Minitab).

Minitab

***Optimal conditions*:**

					C9	Desirability
Solution	**X1**	**X2**	**X3**	**X4**	**Adjust**	**composite**
1	-5.05E-02	-5.05E-02	0.131313	0.131313	21.0126	0.935536

Multiple Response Prediction

Variable	**Configuration**
X1	$-5.05E^{-02}$
X2	$-5.05E^{-02}$
X3	0.131313
X4	0.131313

10. Bibliographic References

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