DOI: http://dx.doi.org/10.21577/1984-6835.20240067

# **Supplementary Information**

# Extraction of Pectin in Seriguela (Spondias 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"

Luiz B. S. Filho,<sup>a,b</sup> (<u>https://orcid.org/0009-0000-2196-0981</u>) Ronaldo C. Coelho,<sup>b,\*</sup> (<u>https://orcid.org/0000-0003-0800-5132</u>) Tiago L. S. Coêlho,<sup>c</sup> (<u>https://orcid.org/0009-0009-8306-7094</u>) Edvani C. Muniz,<sup>d,e</sup> (<u>https://orcid.org/0000-0001-6685-1519</u>) Herbert de S. Barbosa<sup>a</sup> (<u>https://orcid.org/0000-0003-2094-7384</u>)

<sup>a</sup> Universidade Federal do Piauí, Departamento de Química, Grupo de Estudos em Bioanalítica — GEBIO, Zip Code 64049-550, Teresina-PI, Brazil

<sup>b</sup> Instituto Federal do Piauí, Departamento de Formação de Professores, Zip Code 64053-390 Teresina-PI, Brazil

<sup>c</sup> Instituto Federal do Amapá, Departamento de Química, Grupo de Pesquisa em Mineração, Materiais e Meio Ambiente, Zip Code 68909-398 Amapá-AP, Brazil

<sup>d</sup>Universidade Federal do Piauí, Departamento de Química, Zip Code 64049-550, Teresina-PI, Brazil

<sup>e</sup> Universidade Estadual de Maringá, Departamento de Química, Zip Code 87020-900, Maringá-PR, Brazil

\*ronald@ifpi.edu.br

#### 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 welldeveloped 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<sup>®</sup> system (version from 3.0.1), just connect to the website (<u>https://cran.r-project.org/bin/windows/base/old/</u>) 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<sup>®</sup> 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.

← → C a cran.r-project.org/bin/windows/base/old/	8	Ê	☆	*	1	:
Previous Releases of R for Windows						Â
This directory contains previous binary releases of R for Windows.						1
The current release, and links to development snapshots, are available here. Source code for these releases and others is available through the main CRAN page.						1
In this directory:						1
R 4 1.3 (March, 2022) R 4 1 2 (November, 2021) R 1 1 (August, 2021) R 1 1 (May, 2021) R 1 1 (May, 2021) R 1 0 (March, 2021) R 1 0 (Aprit, 2020) R 4 0 1 (June, 2020) R 4 0 1 (June, 2020) R 3 6 (February, 2020) R 3						

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

Name       Last modified       Size       Description         Parent Directory:       -         NEWS R-4.1.1 html       2021-08-10 10:40 101K         R-4.11win case       2021-08-10 10:40 8.5K         SVX-REVISION R-4.1.1       2021-08-10 10:40 46         mdSauntati       2021-08-10 10:40 90         reseace.html       2021-08-10 10:40 90         researce at crank-project.org Port 443		project.org/bin/window	s/base/old/4.1.1/					ē <sub>r</sub>	ê :	☆	*	
Name         Last modified         Size         Description           Parent Directory:         -           NEWS R-4.1.1         2021-08-1010:40         101K           R=A1L-win esse         2021-08-1010:40         55X           SV-REVISION R-4.1.1         2021-08-1010:40         6           stN-REVISION R-4.1.1         2021-08-1010:40         6           mdSum txt         2021-08-1010:40         90           release.hml         2021-08-1010:40         90           rxv-FAQ.hml         2021-08-1010:40         99K	ndex of /bin/	/windows/l	base/old/4	4.1.1								
Parent Directory:       -         NEWS R-4.1.1 kml       2021-08-10 10:40 101K         R-4.1.1-vvin.exe       2021-08-10 11:55 85M         JEADME R-4.1.1       2021-08-10 10:40 8 5K         SVN-REVISION R-4.1.1       2021-08-10 10:40 46         md5um.txt       2021-08-10 11:55 50         release.hml       2021-08-10 10:40 90         rwFAQ.html       2021-08-10 10:40 99K	Name	Last modified	Size Description									
NEWS R=4.1.1.html       2021-08-10 10:40 101K         R=4.1.1-vvin.ese       2021-08-10 11:55 \$\$M         README R=4.1.1       2021-08-10 10:40 \$.SK         SVN-REVISION R=4.1.1       2021-08-10 10:40 \$.46         mdSum.txt       2021-08-10 10:55 \$0         release.html       2021-08-10 10:40 \$.9K         ache Server at cran.r-project.org Port 443	Parent Directory											
R1.1.vvin.exe       2021-08-10 11:55       85M         JEADME.R1.1.1       2021-08-10 10:40       8.5K         SVN.REVISION.R1.1.1       2021-08-10 10:40       46         md5uum.txt       2021-08-10 10:55       50         release.html       2021-08-10 10:40       90         rw.FAQ.html       2021-08-10 10:40       90K         ache Server at cran.r-project.org Port 443	NEWS.R-4.1.1.html	2021-08-10 10:40 1	101K									
README R-1.1       2021-08-10 10:40 8.5K         SUN-REVISION R-1.1       2021-08-10 10:40 46         md5sum txt       2021-08-10 11:55 50         release-limit       2021-08-10 10:40 90         rcv-FAQ.html       2021-08-10 10:40 99K         ache Server at cran.r-project.org Port 443	R-4.1.1-win.exe	2021-08-10 11:55	85M									
SVN-REVISION R-4.1.1 2021-08-10 10:40       46         md5sum.txt       2021-08-10 11:55       50         release.html       2021-08-10 10:40       90         rxv-FAQ.html       2021-08-10 10:40       99K         ache Server at cran.r-project.org Port 443	README.R-4.1.1	2021-08-10 10:40	8.5K									
md5sum_txt       2021-08-10 11:55       50         release.html       2021-08-10 10:40       90         rxv.FAQ.html       2021-08-10 10:40       99K         ache Server at cran.r-project.org Port 443	SVN-REVISION.R-4.1	1 2021-08-10 10:40	46									
release.html         2021-08-10         0:0           rsx-FAQ.html         2021-08-10         0:0         99K           ache Server at cran.r-project.org Port 443         FAQ.html         2021-08-10	md5sum.txt	2021-08-10 11:55	50									
rxv-FAQ.html 2021-08-10 10:40 99K ache Server at cran.r-project.org Port 443	release.html	2021-08-10 10:40	90									
ache Server at cran.r-project.org Port 443	rw-FAQ.html	2021-08-10 10:40	99K									
	vache Server at cran.r-pro	oject.org Port 443										

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.

User Account Control	×
Do you want to allow this changes to your device?	s app to make
R for Windows 4.1.1 S Verified publisher: Jeroen Ooms File origin: Hard drive on this comp Show more details	Setup uter
Yes	No

Figure 3S. Running the file.

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

Select Setup Language						
Select the language to use during the installation.						
	English	~				
OK Cancel						

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.

👸 Setup - R for Windows 4.1.1	-		×
Select Destination Location Where should R for Windows 4.1.1 be installed?			R
Setup will install R for Windows 4.1.1 into the following t	folder.		
To continue, click Next. If you would like to select a different fold C:\Program Files\R\R_4.1.1	er, dick Bro	owse. rowse	
	/		
		/	
At least 2.5 MB of free disk space is required.	/		
< Back	lext >	Ca	incel

Figure 6S. Selecting the installation target location.

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

😥 Setup - R for Windows 4.1.1	-		×
Select Components Which components should be installed?		(	R
Select the components you want to install; dear the components you are ready to continue.	ou do not	want to	_
User installation		~	
Core Files		90.7 MB	
32-bit Files		50.9 MB	
G4-bit Files		57.8 MB	
Message translations	/	8.8 MB	
Current selection requires at least 210.3 MB of disk space.			
< Back Ne	xt >	Can	cel

Figure 7S. Selecting installation components.

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

뤻 Setup - R for Windows 4.1.1	_		×
Startup options Do you want to customize the startup options?			R
Please specify yes or no, then dick Next. Yes (customized startup) No (accept defaults)			
< Back	Next >	Ca	ncel

Figure 8S. Boot options.

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

🕞 Setup - R for Windows 4.1.1	-		Х
Select Start Menu Folder Where should Setup place the program's shortcuts?		(	R
Setup will create the program's shortcuts in the fol	llowing Start M nt folder, click	1enu folder. Browse.	
3		Browse	1
Don't create a Start Menu folder	1	/	
< Back	Next >	Can	icel

Figure 95. Select shortcuts folder.

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

谒 Setup - R for Windows 4.1.1	_		×
Select Additional Tasks Which additional tasks should be performed?			R
Select the additional tasks you would like Setup to perform while Windows 4.1.1, then click Next.	installing R f	for	
Additional shortcuts:			
Create a desktop shortcut			
Create a Quick Launch shortcut			
Registry entries:			
Save version number in registry			
Associate R with .RData files			
< Back	Next >	Ca	incel

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 115(b). Completion of the installation.

### 4. Download and install RStudio

### 4.1. Download

To download RStudio, it is necessary to connect to the website <u>https://www.rstudio.com/products/rstudio/download/</u>, 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 (<u>https://www.rstudio.com/products/rstudio/download/</u>). Also, use the link (<u>https://www.youtube.com/watch?v=rr54DqsJJjY</u>) 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".

당 RStudio Setup			_		×
	Choose Insta Choose the fo	II Location Ider in which to ir	nstall RStudio.		
Setup will install RStudio and select another folde	in the following folc r. Click Next to con	der. To install in a tinue.	different folder,	dick Browse	
Destination Folder	tudio		Br	owse	
Space required: 797.2 N	IB			1	
Space available: 236.9 (	SB		/		
Nullsoft Install System v3.0	8	< Back	Next >	Cano	el

Figure 15S. RStudio installation folder.

🌍 RStudio Setup			_		Х
3	Choose Start M Choose a Start N	<b>lenu Folder</b> 1enu folder for the	RStudio shortcut	s.	
Select the Start Menu fr can also enter a name t	older in which you wou to create a new folder.	ld like to create the	e program's shorto	cuts. You	
Accessibility Accessibility Accessives Administrative Tools AMD Bug Report Tool AMD Software: Adrena ASIO 4ALL v2 BandLab Technologies Battle.net Blender Cakewalk CPUID	lin Edition		/		~
Do not create shortd Nullsoft Install System v3.0	cuts 08	< Back	Install	Cance	el

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.

🕞 RStudio Setup		_		$\times$
	Installing Please wait while RStudio is being installed.			
Extract: pdf.worker.js.map	<b>b</b>			
Show details				
Nullsoft Install System v3.08 -				
	< Back Next	>	Cance	2l

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).

R Choose R Installation						
RStudio requires an existing installation of R. Please select the version of R to use.						
Use your machine's default 64-bit version of R     Use your machine's default 32-bit version of R						
Choose a specific version of R:						
[64-bit] C:\Program Files\R\R-4.1.1 [32-bit] C:\Program Files\R\R-4.1.1	*					
	Ŧ					
You can also customize the rendering engine used by RStudio. Rendering Engine: Auto-detect (recommended) ♥ Browse OK Cancel						

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 <u>https://cran.r-project.org</u>, 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("packagename", 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.

File	Files Plots Packages Help Viewer								
Ø	Install O Update	Description							
User	Library								
	abind	Combine Multidimensional Arrays							
	affy	Methods for Affymetrix Oligonucleotide Arrays							
	affyio	Tools for parsing Affymetrix data files							
	agricolae	Statistical Procedures for Agricultural Research							
	albatross	PARAFAC Analysis of Fluorescence Excitation-Emission Matrices							
	AlgDesign	Algorithmic Experimental Design							
	alr4	Data to Accompany Applied Linear Regression 4th Edition							
	ALS	Multivariate Curve Resolution Alternating Least Squares (MCR-ALS)							
	alsace	ALS for the Automatic Chemical Exploration of mixtures							
	ALSCPC	Accelerated line search algorithm for simultaneous orthogonal transform of several positive definite symmetric matrices to nearly diagonal form.							
	ALSM	Companion to Applied Linear Statistical Models							
	ach	David Scott's ASH Routines							

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.

Install Packages	
Install from: Repository (CRAN)	<ul> <li>Configuring Repositories</li> </ul>
Packages (separate multiple v rsm	vith space or comma):
Install to Library: C:/Users/luizb/OneDrive/Do	cumentos/R/win-library/4.0 [Defaul: ▼
Install dependencies	
_	Install Cancel

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.

🗷 RS	tudio									
File	Edit	Code	View	Plots	Session	Build	Debug	Profile	Tools	Help
۰ ،	OR	• 🕋			🔿 Go te	o file/fun	ction	= •	Addins	•
0	Untitla	418 0								
	ontitle					-226	-			
			U Sou	irce on Sa	ave 🔍					
	2									
	3 jir	stall	.pack	ages ('	'rsm",	depen	dencies	5 = TRU	E)	
	•						~			
							<b>~</b>			

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 - <u>https://cran.r-</u> 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.

$\leftrightarrow$ $\rightarrow$ C $\$ a cran.r-project.org/bin/windows/Rtools/history.html		ම ය 🛪 🛱 🗖 🖣	<b>)</b> :						
R for Windows Build Tools Archive									
This document is a collection of resources that were used in the past for building packages for R under Microsoft Windows, and for building R itself (version 1.9.0 or later). The original collection was put iogether by Prof. Brian Ripley and Duncan Murdoch; it is currently maintained by Jeroen Ooms.									
Instructions for the current version of R can be found on the <u>Risols homepage</u> . The authoritative source of information for tools to work with the current release of R is the "R Administration and Installation" manual. In particular, please read the " <u>Windows Toolset" appendix</u> .									
Rtools Downloads	Rtools Downloads								
Some of the tools are incompatible with obsolete versions of R. We maintain one actively update Rtools with the latest release of R.	Some of the tools are incompatible with obsolete versions of R. We maintain one actively updated version of the tools, and other "frozen" snapshots of them. We recommend that users use the latest release of R tools with the latest release of R								
The current version of this file is recorded here: <u>VERSION.txt</u> .									
Download	R compatibility	Frozen?							
Windows 64-bit: <u>ttools40-x86_64.exe</u> (recommended: includes both i386 and x64 compilers) Windows 32-bit: <u>ttools40-i686.exe</u> (i386 compilers only)	R 4.0 and up	No							
Rtools35.exe	R 3.3.x to 3.6.x	Yes							
Rtools34.exe	R 3.3.x to 3.6.x	Yes							
Rtools33.exe	R 3.2.x to 3.3.x	Yes							
Rtools32.exe R 3.1 x to 3.2 x Yes									
Rtools31.exe	R 3.0.x to 3.1.x	Yes							
Rtools30.exe	R > 2.15.1 to R 3.0.x	Yes							
Rtools215.exe	R > 2.14.1 to R 2.15.1	Yes							
Rtools214.exe	R 2.13.x or R 2.14.x	Yes							
Rtools213.exe	R 2.13.x	Yes							
lin tata	In a ca								

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.

User Account Control X						
Do you want to allow th changes to your device?	is app to make					
Rtools Setup Verified publisher: Jeroen Ooms File origin: Hard drive on this com Show more details	puter					
Yes	No					

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.

ng Rtool		×
ng Rtool		
ng Rtoo	Ę	
ng Rtoo		
	is, then	
	Cano	el
		Cano

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.

R Setup - Rtools version 4.0	-		×
Ready to Install Setup is now ready to begin installing Rtools on your computer.			
Click Install to continue with the installation, or click Back if you wan change any settings.	it to revie	w or	
Destination location: C:\ytools40		/	
Additional tasks: Save version information to registry			
<		>	/
< Back In	stall	Ca	ncel

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

R Setup - Rtools version 4.0	_		×
Installing			
Please wait while Setup installs Rtools on your computer.			
Extracting files			
C: \rtools40 \mingw32 \i686-w64-mingw32 \indude \dxva2api.idl			
		0	ncel
		Ca	SI ICEI

Figure 32S. "Rtools" package installation progress.



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

## **Tables and Figures**

Table 1S.	Fractional	factorial	design 2 <sup>5-</sup>	<sup>1</sup> annlied in	nectin	extraction	from	seriguela	neel
Table 13.	riactional	lactonal	uesigniz	applied in	pecun	extraction	110111	Seligueia	peer.

Factors		Un	its		Coded levels	5
				-1	0	1
рН (х1)		-		1.5	2.1	2.7
Temperature	(x <sub>2</sub> )	•	С	60	70	80
Time (x₃)	Time (x₃)		in	15	30	45
Power (x <sub>4</sub> )		V	V	1	3	5
Ratio (x₅)		g n	1L <sup>-1</sup>	15	22.5	30
Exp.	<b>X</b> 1	<b>X</b> 2	<b>X</b> 3	<b>X</b> 4	<b>X</b> 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

Factors		Units	Coded levels			
			-1	0	1	
рН (х1)		-	2	2.35	2.7	
Temperature	e (x2)	°C	70	75	80	
Time (x₃)		min	25	35	45	
Power (x <sub>4</sub> )		W	2	3.5	5	
Exp.	<b>X</b> 1	<b>X</b> 2	<b>X</b> 3	<b>X</b> 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	

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



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

		Interaction plot		
04 •1 x1 € •1 x1				<b>∳</b> ♥
15 40	⊽-1 •1 x2	<b>*</b> *********	<b>*</b> *	<b>∲</b> ····••
₹ <b>1</b> 2 <b>1</b> 2 <b>1</b> 0	÷	⊽-1 •1 <sup>X3</sup>		♦
₹	<b>∲</b> <del>\</del>	\$₽	⊽-1 ◆1 X4	∲∳
15 40	••	<b>*</b>	•	∇-1 •1 x5

Figure 34S(b). Interactions graph.

<pre>call: FrF2(nruns = 16, ncenter = 3, nfactors = 5, factor.names = c("x1", "x2", "x3", "x4", "x5"), randomize = F, alias.info = 3)</pre>
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
Sgenerators [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 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 $-1 - 1 - 1 1$ 10 $1 - 1 - 1 1$ 11 $-1 1$ 11 $-1 1$ 12 $1 1 - 1 1$ 13 $-1 - 1 1$ 14 $1 - 1 1$ 15 $-1 1 1 1$ 15 $-1 1 1 1$ 16 $1 1 1 1$ 17 $0 0 0 0 0$ 18 $0 0 0 0 0 0$ 19 $0 0 0 0 0$ 19 $0 0 0 0 0$ 19 $0 0 0 0 0$

Figure 355. Summary table of the structure of fractional factorial design 2<sup>5-1</sup>.



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</pre>
```

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	рн	te	tp	p₩		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	I.	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	Ĭ.	20.588
10	1.0	0.100	-0.626	0.004	0.774	2.38500	71.870	35.04	4.6610	1	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</pre>
```

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)

```
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)
```

#### # Figure 41S(D)

#### # Figure 41S(E)

```
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)



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.<sup>1</sup>

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.<sup>2</sup> 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.<sup>3</sup>

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)

Coefficients		
Design Expert		
25.80625		
3.61625		
0.83125		
2.28375		
2.6575		
-2.96125		
3.0325		
-0.60292		

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

Table 4S. Anova table (Design Expert).

ANOVA for selected factorial model								
Analysis of variance table [Partial sum of squares - Type III]								
	Sum of		Mean	F	p-value			
Source	Squares	df	Square	Value	Prob > F			
Model	704.1779	6	117.363	84.7356	< 0.0001	significant		
А-рН	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<sup>2</sup> 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 \* pH + 0.0831 \* Temperature. + 0.843 \* Time- 3.978 \* Power - 0.329 \* pH \* Time + 2.527 \* pH \* Power (15)

Table 55. Summary table of the significance of the estimated coefficients (Minitab)

Factor	Coefficients (Design Expert)
Intercept	25.711
А-рН	3.616
<b>B-Temperature</b>	0.831
C-Time	2.284
D-Power	2.657
AC	-2.961
AD	3.033
Central point	-

ANOVA for selected factorial model								
Analysis of variance table [Partial sum of squares - Type III]								
Sum of Mean F p-value								
Source	Squares	df	Square	Value	Prob > F			
Model	719.291	15	47.953	138.27	< 0.001			
Linear	418.839	5	83.768	241.54	0,000			
А-рН	209.236	1	209.236	603.03	< 0.0000			
<b>B-Temperature</b>	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						

# Table 6S. Anova Table (Minitab)

# Model Summary

S	R <sup>2</sup>	R²(aj)	R <sup>2</sup> (pred)	
0.588898	99.86%	99.13%	0.00%	

## Regression Equation in Uncoded Units

$$C11 = 25.711 + 3.616 X1 + 0.831 X2 + 2.284 X3 + 2.657 X4 + 0.363 X5 + 0.269 X1 * X2 - 2.961 X1 * X3 + 3.033 X1 * X4 - 0.385 X1 * X5 + 0.199 X2 * X3 - 0.175 X2 * X4 - 0.222 X2 * X5 - 0.128 X3 * X4 + 0.668 X3 * X5 + 0.106 X4 * X5$$
(25)



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

	Sum of			Mean	F	p-value	
Source	Squares	df		Square	Value	Prob > F	
Model	50.00046		14	3.571461	21.48901	< 0.0001	significant
А-рН	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 <sup>2</sup>	26.96506		1	26.96506	162.2452	< 0.0001	
B <sup>2</sup>	5.485986		1	5.485986	33.00845	< 0.0001	
C <sup>2</sup>	18.21821		1	18.21821	109.6166	< 0.0001	
D <sup>2</sup>	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				

Table 75. ANOVA summary table and regression coefficients.

The model as well as the terms A, C, D, AD, BC, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>, D<sup>2</sup> are significant. The "lack of fit" was not significant relative to the pure error.

### 9.2. Optimization Minitab

PRESS

12.35003

	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	50.0811	14	3.5772	22.49	< 0.000	
А-рН	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 <sup>2</sup>	26.5938	1	26.5938	167.21	< 0.000	
B <sup>2</sup>	5.3192	1	5.3192	33.45	< 0.000	
C <sup>2</sup>	18.8357	1	18.8357	118.43	< 0.000	
D <sup>2</sup>	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-9	Squared	0.95	5534	
Mean	18.62793	Ad	j R-Squared	0.91	1068	
C.V. %	2.188518	Pro	ed R-Squared	0.76	3985	

 Table 8S. ANOVA summary table and regression coefficients.

The R<sup>2</sup> 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.

15.29102

Adeq Precision

Factor	Coefficients					
	Design Expert	Minitab				
Intercept	20.9538	20.954				
А-рН	-0.30442	-0.304				
<b>B-Temperature</b>	-0.10942	-0.109				
C-Time	0.464417	0.436				
D-Power	0.280083	0.308				
АВ	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 <sup>2</sup>	-2.0389	-2.025				
B <sup>2</sup>	-0.91965	-0.906				
C <sup>2</sup>	-1.6759	-1.704				
D <sup>2</sup>	-0.9864	-1.015				

**Table 95.** Comparative table of coefficients (Design Expert x Minitab).

## Model equation:

## Design expert

$$Y\% = -264.486 + 68.715 * pH + 5.056 * Temperature + 0.601 * Time + 3.026$$
  
\* Power + 0.0819 \* pH \* Temperature - 0.095 \* pH \* Time  
+ 1.664 \* pH \* Power + 0.012 \* Temperature \* Time - 0.0452  
\* Temperature \* Power - 8.367E - 003 \* Time \* Power - 16.644  
\* pH<sup>2</sup> - 0.037 \* Temperature<sup>2</sup> - 0.0168 \* Time<sup>2</sup> - 0.438  
\* Power<sup>2</sup>
(35)

### Minitab

$$C9 = 20.954 - 0.304 X1 - 0.109 X2 + 0.436 X3 + 0.308 X4 - 2.025 X1 * X1$$
  
- 0.906 X2 \* X2 - 1.704 X3 \* X3 - 1.015 X4 \* X4 + 0.143 X1 \* X2  
- 0.332 X1 \* X3 + 0.873 X1 \* X4 + 0.580 X2 \* X3 - 0.339 X2 \* X4  
- 0.041 X3 \* X4 (45)



**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 495. 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 <sup>-02</sup>
X2	-5.05E <sup>-02</sup>
ХЗ	0.131313
X4	0.131313

## **10. Bibliographic References**

1. Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). Response Surface Methodology (3th ed.). Wiley. [Crossref]

2. Neto, B. B., Scarminio, I. S., & Bruns, R. E. (2010). Como fazer experimentos (4th ed.). Bookman. [Crossref]

3. Lenth, R. v. (2009). Response-Surface Methods in R, Using rsm. Journal of Statistical Software, V. 32,

7. [Crossref]