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The Effects of Alpha-amylase, Ascorbic Acid and Low Pressure during Mixing on the Texture of Bread Crumb

Image Analysis and Measurements of Physical Texture

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Seinäjoen ammattikorkeakoulu
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Thesis Abstract

Faculty: School of Technology

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Author: Charlotta Tuomisto

Title of thesis: The Effects of Alpha-amylase, Ascorbic Acid and Low Pressure during Mixing on the Texture of Bread Crumb

Supervisors: Pasi Junell, Tim Van Dyck

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The objective of the thesis was to research how different factors have an influence on the structure of bread. The variables used in this study were ascorbic acid, alpha-amylase and the use of vacuum when mixing dough. Viscoelastic properties and porous structure of bread were studied.

The main objective of the study was to build a foundation for a wider research on the structure of bread. This was accomplished by creating a frame for a model, where the microstructural and mechanical properties of bread can be compared.

This particular study was a quantitative empirical study, where several computer programs were utilized. The limit values and the related measurements of the research were determined. Calculations were executed mainly by measurement devices and computer programs. The research took place under laboratory circumstances.

The research revealed that related factors had an influence on several properties studied and had also joint effects. Due to the research design, the effects of the factors studied cannot be differentiated from each other. It was evident, though, that the porous structure of the bread and volume were especially sensitive for the presence of all the studied factors. Of all the factors, alpha-amylase had the most extensive influence on the studied qualities and affected on the viscoelastic characteristics of the bread. Whereas pressure had an effect only on the viscoelasticity and ascorbic acid on the viscous properties.

Keywords: alpha-amylase, ascorbic acid, vacuum, bread, rheology, texture

SEINÄJOEN AMMATTIKORKEAKOULU

Opinnäytetyön tiivistelmä

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Tämän opinnäytetyön tarkoituksena on ollut selvittää eri tekijöiden vaikutusta leivän rakenteeseen. Käytettyjä muuttujia olivat askorbiini happo ja alpha-amylaasi. Lisäksi yhtenä muuttujana oli paine taikinaa vaivatessa. Leivästä tutkittiin sen viskoelastisia ominaisuuksia sekä huokoista rakennetta.

Tutkimuksen tarkoituksena on toimia suuntaa antavana pohjatyönä laajempaan leivän rakennetta käsittelevään tutkimukseen. Tavoitteena on luoda kehykset mallia varten, missä mikrorakenteen ominaisuuksia ja leivän mekaanisia ominaisuuksia voidaan verrata. Opinnäytetyön on selvittää onko kyseessä olevasta tutkimuksesta havaittavissa ominaisuuksia, joita kannattaisi tutkia lisää ja auttaa mallin rakentamisessa.

Kyseessä oli kvantitatiivinen empiirinen tutkimus, jossa käytettiin useita valmiita tietokoneohjelmia hyödyksi. Tutkimuksen raja-arvot päätettiin itse ja mittaukset päätettiin itse, mutta mittauslaitteiden omat sekä muut ohjelmat suorittivat pääosin laskutoiminnot. Tutkimus suoritettiin laboratorio-olosuhteissa.

Tutkimustuloksista havaittiin, että tekijöillä oli useampaan mitattavaan ominaisuuteen vaikutus ja tekijöillä oli myös yhteisvaikutuksia. Tutkimus asettelusta johtuen, tekijöiden vaikutuksia ei voi täysin eriyttää toisistaan. Havaittavissa kuitenkin oli, että leivän huokoinen rakenne ja koko ovat erityisen herkkiä muuttujille. Alpha-amylaasilla oli tekijöistä laajin vaikutus ja se vaikutti leivän viskoelastiseen luonteeseen. Paine puolestaan vaikutti vain elastiseen luonteeseen ja askorbiini happo viskoosisiin ominaisuuksiin.

Avainsanat: alfa-amylaasi, akorbiini happo, vakuumi, leipä, reologia, rakenne

TABLE OF CONTENTS

Tables and figures

Abbreviations

μm	micrometer
A	pressure
B	ascorbic acid
C	alpha-amylase
Ca^{2+}	calcium ion
cm	centimeter
CT	computed tomography
DPI	dots per Inch
EU	enzyme Units
g	gram
kg	kilogram
mBar	millibar, 1 bar = 100 000 Pa
mm	millimeters
mm/sec	millimetres per second
N	force
^{o}C	Celsius degrees
Pa	Pascal, N/m^2
pH	activity of the hydrogen ion
ppm	parts per million
R^2	R-square

1 INTRODUCTION

1.1 Subscriber

The study was made in Lab4Food which belongs to Department of Biosciences in Katholieke Hogeschool Kempen which has recently changed its name to Thomas More Kempen. Lab4Food focuse on food safety, rheology of food and food structure. It is located in Geel, Belgium. (KHKempen. [Referred:2.1.2013].) This study was ordered by Ph.D. student Tim Van Dyck and it works as a framework for his larger study.

1.2 Objective and research steps

The purpose of the study was to survey in outline three different variables what comes to baking bread and to measurements of texture. The three variables that were used in this study were alpha-amylase, ascorbic acid and low pressure during the mixing. The study was planned with Design-Expert 8.0.7.1. The program offers statistical tools and helps to create a study plan and also helps to process the results. (Stat-Ease, Inc. [Referred:31.1.2012].) The measurements were done by scanning, texture analyser and rheometer. One objective was also to find a correlation between different measurements and results. The thesis also works as an internship report.

The research was a part of a larger study related to the microstructural properties of bread crumb to the sensorial perception with consumers. Research on the quality aspects of bread has influenced large markets. The industry has a very large interest in what comes to quality and texture of bread. Consumers are more aware nowadays, and they demand new and innovative products. The immediate goal of the work presented here is to provide a model bread with varying texture properties and a protocol to measure the mechanical properties of bread crumb. Both of these elements, in combination with later microstructural analysis, will provide a backbone for mechanical models for relating microstructural properties to the macroscopic mechanical behaviour of breadcrumb. X-ray visualization and

CT imaging to bread among mechanical properties measurements have already been done (Wang, Austin & Bell 2011, 204-207).

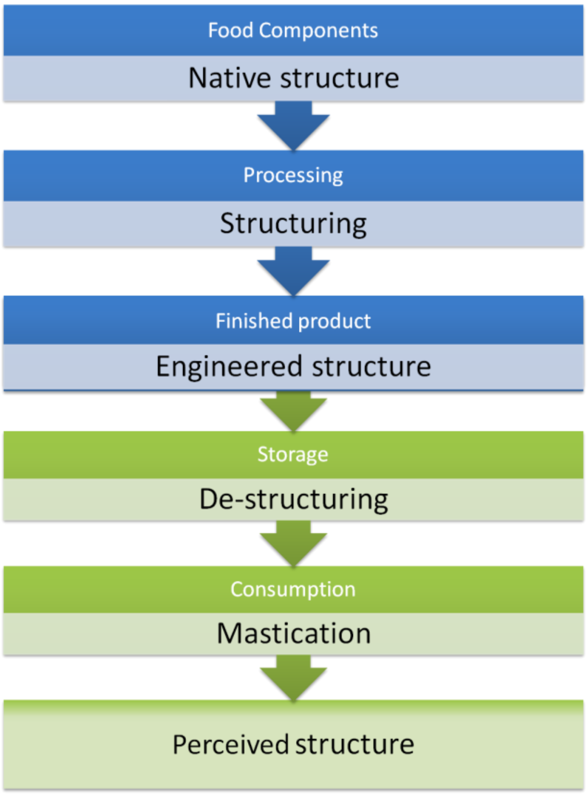


Figure 1. Stages of food stuffs structures development.

Figure 1 presents, according to Aguilera and Stanley (Aguilera, Stanley & Baker 2000, 8.), how the structure of a food product develops. This study focuses on the blue part of the diagram and it also follows the same order. First there are ingredients and in outline their biochemistry that influences on the structure. Second there are baking methods and also their influence on the final product. Then there is the last part of the diagram which is the measurements and results of the final products.

2 MATERIAL AND METHODS

2.1 Ingredients and recipe

In this study the basic baking process was kept very simple. Hence, there were fewer factors that could have influenced the research results. For example, butter or margarine was cut off from the ingredients because the quality of fats could have influenced the results (Belitz & Crosch 1999, 664). A small amount of liquid margarine was used for rubbing the baking tins.

As ingredients for every batch, 1000g of flour, 600g of water, 30g of dry yeast and 16g of salt were used. The amount of alpha-amylase varied between 0-8000 EU and the amount of ascorbic acid varied between 0-80 ppm per batch.

Wheat flour. The flour was manufactured in Nv Bloemmolens Van Geel Sa. The flour contained extra gluten but there were no additives in it. Wheat (*Triticum aestivum*) kernel itself also contains gluten. Gluten gives dough cohesive and elastic properties. It is composed of glutenin and gliadin. (Matz 1992, 4-6). According to a farinograph test, this flour can be called “strong” flour because it resists stretching and is really elastic (Coultate 2009, 199).

Wheat consists of a huge amount of different enzymes. However, starch-digesting enzymes are the most relevant ones in this case. Alpha-amylase is one of these starch-digesting enzymes and it plays a major role in process of carbohydrase enzyme, because enzymes in yeast are unable to break down the starch. (Matz 1992, 10-11.)

Wheat kernel consists of small amounts of mono- and disaccharides which are fructose, glucose, galactose, sucrose, difructose and maltose. The starch polymer is made up of glucose and it is the biggest fraction in the wheat kernel. The starch has a capability to absorb a high amount of water. (Matz 1992, 6-7.) Starch is composed of two glucans: amylase and amylopectin. Wheat starch granule contains 26-31 % amylase. It is lenticular and polyhedral shaped and its diameter is 2-38 µm. Crystallinity percentage is 36. (Belitz & Crosch 1999, 297,278.)

Wheat flour has relatively a low content of fats. Most of the fatty acids in flour are polyunsaturated. There are also saturated and monounsaturated fatty acids. (Fineli [Referred 16.10.2012].) The total content of fiber in white wheat flour is approximately 3,6g/100g. Half of the fiber is insoluble. (Coultate 2009, 75.)

Water and salt. Pure water at room temperature was used in this study. Water has a capability to dissolve materials. The salt content has an influence on fermentation and gluten strength. Sodium chloride is commonly used in foodstuff because it gives a pure salty taste. (Matz, 1992, 123-124,134.) Water is also needed for enzymatic reactions (Belitz & Grocsh, 1999, 136).

Yeast. In yeast, fermentation yeast cells (*Saccharomyces cerevisiae*)~~,~~ generate carbon dioxides from glucose or fructose. In the following reaction equation glucose hydrolyzes to ethyl alcohol and carbon dioxide:

$$C_6H_{12}O_6 \leftrightarrow 2\ C_2H_5OH + 2\ CO_2$$

The enzymes that are present in the yeast can hydrolyze sucrose and maltose. Nevertheless, those enzymes are unable to break down starch to glucose residues. Carbon dioxide leavens the dough and ethyl alcohol gives a characteristic aroma to the product. (Matz 1992.)

For good growth yeast needs warmth, moisture, air, carbohydrates and acids. For *Saccharomyces cerevisiae*, the maximum growth temperature range is between 35-45 ^{o}C. Water is vital for metabolism of yeast cells. (Walker 1998, 144.)

Alpha-amylase. Alpha-amylase is able to hydrolyze starch, glycogen and other 1, 4-alpha-glucans. For activation it needs Ca^{2+} ions. It gives the bread more volume but it has negative effect on pores and structure. (Belitz & Grosch 1999, 313,674.)

Alpha-amylase used in this study was a liquid enzyme preparation called Novozymes Termemyl. It is produced by a genetically-modified strain of *Bacillus licheniformis*. This enzyme has good heat stability. The purpose of the alpha-amylase is to hydrolyze linkages between amylose and amyloektin. The optimum activity can be reached at 90 ^{o}C when the pH is 7. (NCBE [Referred: 29.12.2012].)

Ascorbic acid. Ascorbic acid is used as an additive in foodstuff and its E-code is E 300. Ascorbic acid has not got a maximum limit of usage in foodstuff and it can be used on quantum satis –principle. (R 1130/2011) Quantum satis –principle is defined in EU regulation (R 1333/2008) as follows:

> "'quantum satis' shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled."

Ascorbic acid can also be considered a vitamin-C. It is used in baking to eliminate oxidizing process. (Coultate 2009, 205.) Ascorbic acid lowers the requirement of mixing. It increases the strength of the dough and the volume of the bread. (Belitz & Grosch 1999, 670.)

2.2 Baking process

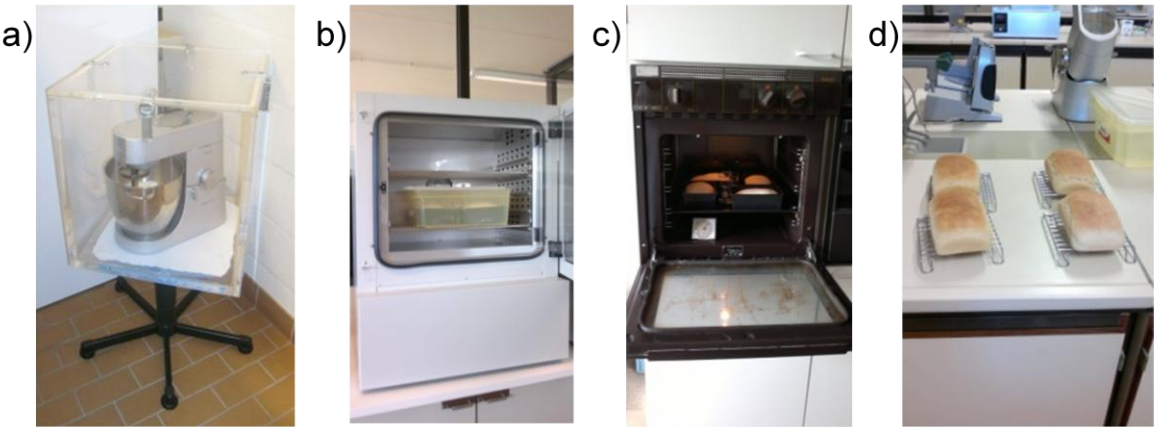


Figure 2. Photos from baking process: a) mixing in the airtight box, b) leavening in the incubator, c) baking in the oven and d) leavening at room temperature.

The dough was mixed for 10 minutes inside an airtight box in Kenwood Mixer at speed one. The dough was divided to four tins after mixing. The aim was to minimize the influence of a baker’s actions to the dough so at this point there was no kneading by hands.

Mixing makes dough homogenous. During the mixing hydration happens and flour polymers absorb a large amount of water. Mechanical work develops gluten proteins by breaking starch granules into smaller residues and makes the dough a cohesive mass. This viscoelastic mass has encapsulated air. The amount of air is related to the formation of foam structure during leavening and baking. (Scanlon & Zghal 2001, 843.)

In addition, the development of gluten has also a big influence on the matter. Especially gliadin molecules are considered to have an influence on making dough viscoelastic. Glutenin for instance needs more mixing than gliadin and it makes dough stronger. It gives higher volume to bread. (Coultate 2009, 205.)

Pressure during the mixing varied between 50-500 mBar. Mixing in vacuum has a decreasing effect on the number of gas cells. (Scanlon & Zghal 2001, 844.) Vacuum was made by pumping air out of the box with a vacuum pump. The pressure was controlled with a manual valve and pressure gauge.

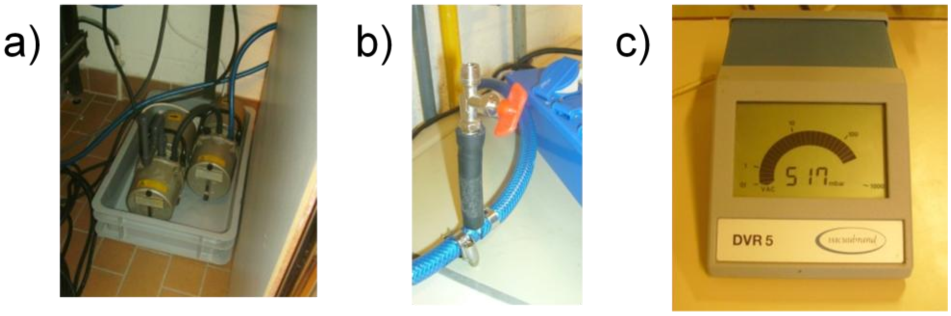


Figure 3. Photos from how to vacuum was created an airtight box; a) a vacuum pump, b) a control valve and c) a pressure gauge.

Leavening was conducted in an incubator at the temperature of 30 ^{o}C degrees for one hour. The main purpose of leavening is the fermentation of the dough and thus the growth in the volume of bread. Gas cells start to expand and some of them coalescence. Starch has a reducing effect on that. (Scanlon, & Zghal 2001, 843-844)

The four breads were in the oven at 200^{o}C degrees for 25 minutes. In an ordinary oven, heat is transferred to bread by air convection and in the bread by conduction. (Hautala & Peltonen 2009, 167.) The rest of the fermentation happens in the oven until temperature rises too high for yeast (Belitz & Crosch 1999, 677). The liquid phase changes to solid and after that bread is unable to expand anymore (Scanlon & Zghal 2001, 846). Ascorbic acid loses its vitamin activity in the oven. (Coultate 2009, 205.) Starch starts to gelatinize at 53-65 ^{o}C temperature (Belitz & Crosch 1999, 278).

Breads formed and cooled in room temperature for approximately two hours. The temperature of the samples has a big influence to the rheological measurements (Krause 2005, 31).

Samples were taken from cooled breads as soon as possible. As mentioned earlier, four breads were baked from each batch. From the middle of the bread three slices were cut with a knife. The bread was placed in a cutting frame so that same sized samples were easy to get. Each sample was three centimetres thick. The middle slice was always used for taking scanning, and from the other two slices each, one cylinder sample was taken. The middle slice was cut with bread slicer to thinner slices (figure 4). The cylinder shaped samples were taken with a drill and the diameter of the samples was 35cm. The samples were used in Texture Analyser’s compression test. After a compression test, the samples were measured with the rheometer. Crust structure was not included in the measurements of physical structure.

Figure 4. Photos from used equipments for taking samples.

2.3 Adjustments of the study

The study plan concluded 32 runs. In the study plan, three factors were named with letters A, B and C where A was pressure, B was ascorbic acid and C was alpha-amylase. The design type was central composite and the model was quadratic so it was possible to evaluate also the quadratic behaviour of the results and not only linear.

2.4 Texture analysis

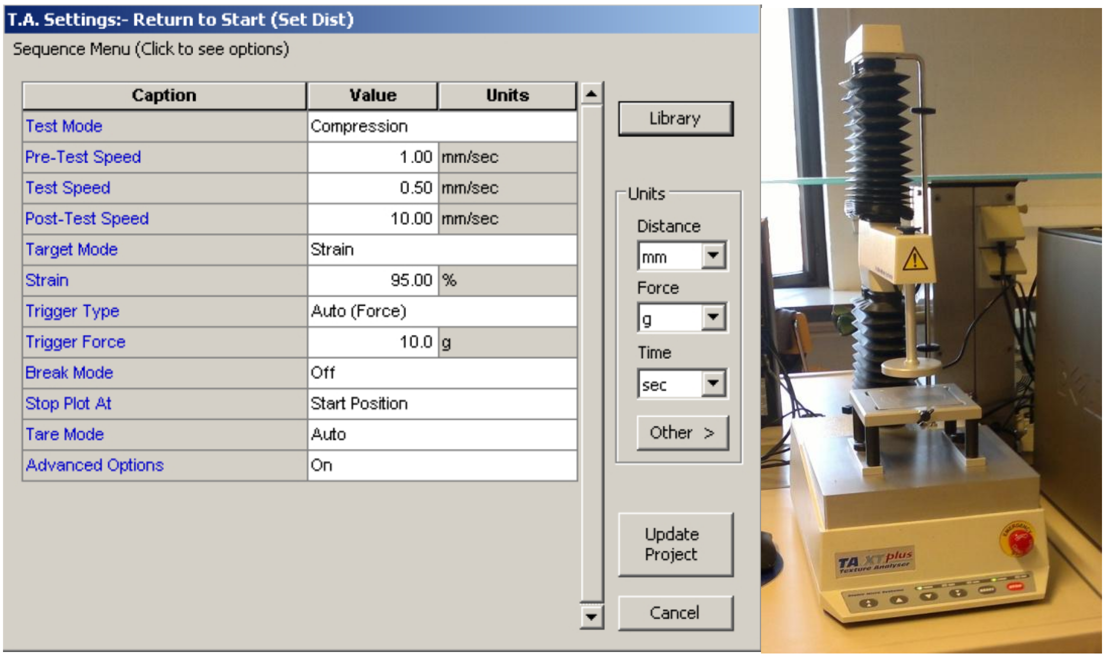
There is no exact definition for texture. Texture is not related to taste or odour. It includes a group of properties. Those are mechanical and rheological properties.

> “The textural properties of a food are that group of physical characteristics that arise for the structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration, and flow of the food under a force, and are measures objectively by functions of mass, time and distance.” (Bourne 2002, 14-15.)

Rheology of texture includes Young’s modulus, shear modulus, Poisson’s ratio, viscosity and loss compliance. (Bourne 2002, 16.)

2.4.1 Measurements of physical texture

A compression test was done with Texture Analyser: TA-XT Plus. Test speed was 0,50 mm/sec and trigger force was 10g. With a low trigger force it is possible to get more exact results, but in this case 10g was found to be the best, because otherwise in some cases the machine started to measure strain before it even touched the sample. A 50kg measuring head was used in this case so the compressing capacity was relatively high. The strain value was set to 95 %. From the compression test results it was possible to find out the stress and strain rates of the crumb, and thereby get results of yield stress, yield strain, collapse stress, collapse strain, elasticity of crumb and elasticity of solid phase. MATLAB® was used for the calculations.



T.A. Settings:- Return to Start (Set Dist)

Sequence Menu (Click to see options)

Caption	Value	Units
Test Mode	Compression	
Pre-Test Speed	1.00	mm/sec
Test Speed	0.50	mm/sec
Post-Test Speed	10.00	mm/sec
Target Mode	Strain	
Strain	95.00	%
Trigger Type	Auto (Force)	
Trigger Force	10.0	g
Break Mode	Off	
Stop Plot At	Start Position	
Tare Mode	Auto	
Advanced Options	On	

Units

Distance: mm

Force: g

Time: sec

Figure 5. Settings of Texture Analyser. (1)

Young's modulus of elasticity measures the stiffness of solid material. It is a ratio of stress and strain when the material is compressed or extended.

$$E = \frac{\text{Tensile stress}}{\text{Tensile strain}} = \frac{\frac{F}{A}}{\frac{\Delta L}{L}} \qquad (1)$$

E = Young's modulus

F = applied force perpendicular to the area defined by stress

A = cross-sectional area of test material

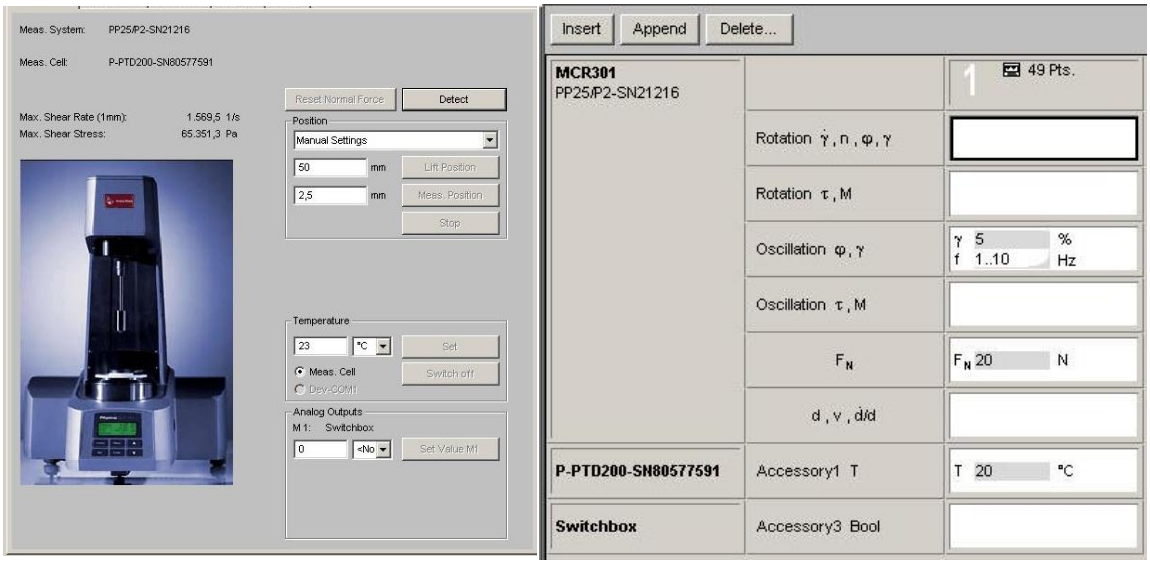
L = length of the test sample

ΔL = change in the length resulting from the application of force

The modulus should only be used for elastic material. (Bourne 2002, 60,68; Young & Freedman 2002, 339.)

Elastic material changes its shape during the stress but when the stress stops, it returns to its original shape. Unlike elastic material, viscous materials change permanently through the influence of stress. Viscoelastic materials are combinations of those properties. (Bourne 2002, 96-97.)

Rheometers settings were set so that the maximum shear rate was 1569.5 per second and the maximum of shear stress was 65351.3 Pa. The measurements were done in 20°C temperature. The height difference between the platform and a tool during the measurements was 2.5 mm, and the measurement surfaces that touched the sample were rough. The force used in the measurements was 20N.



Meas. System: PP25/P2-SN21216
Meas. Cell: P-PTD200-SN80577591
Max. Shear Rate (1mm): 1.569,5 1/s
Max. Shear Stress: 65.351,3 Pa

Position
Manual Settings
50 mm
2,5 mm

Temperature
23 °C
● Meas. Cell
○ Dev-COM1

Analog Outputs
M 1: Switchbox
0 <No

MCR301 PP25/P2-SN21216		1 49 Pts.
	Rotation $\dot{\gamma}$, n, φ, γ	
	Rotation τ, M	
	Oscillation φ, γ	γ 5 % f 1..10 Hz
	Oscillation τ, M	
	F_N	F_N 20 N
	d, v, ḋ/d	
P-PTD200-SN80577591	Accessory1 T	T 20 °C
Switchbox	Accessory3 Bool	

Figure 6. Settings of rheometer measurements.

From the results, Rheometers program calculates a loss and storage modulus of the solid phase of the crumb. Storage and loss modules are parts of dynamic modulus. A storage modulus represents in this case the refundable energy of a compressed crumb and, hence, elastic part. A loss modulus on the other hand represents the energy that is lost under stress. (Mezger 2002.)

2.4.2 Image analysis

A visual texture was studied by scanning thin slices of the bread with HP ScanJet 5300C. The resolution of the pictures was 600 DPI. The pictures were manipulated with a photo editor, so that the solid phase of the bread appeared black and the gas cells appeared white. With these pictures, it was also possible to solve the surface of the slices, the numbers of pores, the surface of pores, the apparent porosity and the surface of solid phase also with MATLAB®. In figure 7, there is an example of the slices with the image analysis. However, the image does not correspond to the actual images completely, because the picture in the figure had to be modified for printing related reasons. Still, the porous structure can be seen in the picture.

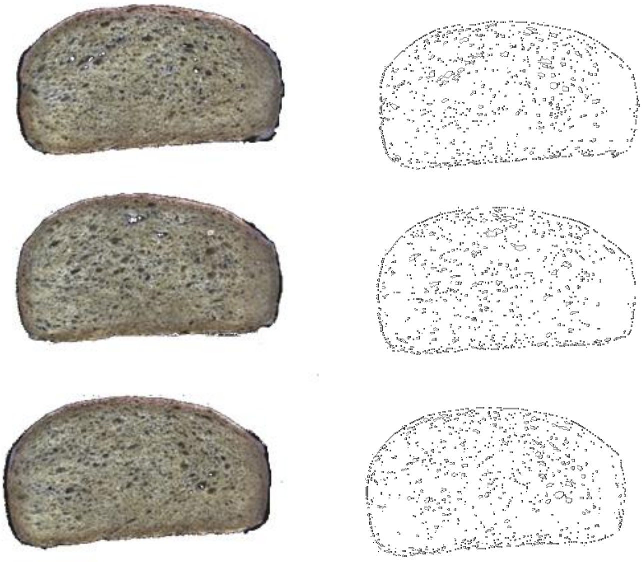


Figure 7. Picture of scanned slices before and after photo editing.

MATLAB$^{®}$ is a product of The MathWorks Inc. It can be used for numerical computation, visualization and programming. It is possible to code individual programs and create models, analyze data etc. (The MathWorks, Inc [Referred: 2.2.2013].) When the results of the compression test were analyzed using MATLAB, the code gave ready results and also made two types of diagrams. An example of them (run 16) can be seen in attachment 3.

3 RESULTS

The study plan included 32 runs, but the batch which included 8000 EU alpha-amylase, no ascorbic acid, and was mixed in 50mBar vacuum, was too sticky for measuring. Thus, only 31 measurements were done. Significance and fidelity calculations were made with the program. For that, the program used ANOVA method, and from those calculations it was possible to delete the factor or the combination of factors which were not significant. The study plan and the results of the measurements can be seen in attachment 1 and design summary in attachment 2.

Because there were three different factors in this study, the program used 3-way ANOVA. ANOVA is abbreviation of ANalysis Of VAriances. ANOVA is based on the equality of factional expectation values when the fundamental population is divided into several groups. Fundamental sets expectation values' equality is tested by testing variances equality with F-tests. (Mellin, 2005 [Referred: 6.2.2013].) R^2 is used for minimizing the problem of regression estimation, and it is a model of minimizing a risk based on empirical data (Vapnik 1998, 26). With a regression analysis it is possible to evaluate how the results fit in to the line. The smaller the R-squared value, the more points are further from the line. If the R-squared gets value 1, all the points fit in the line. (Read, 1998 [Referred 6.2.2013].)

3.1 Yield Stress and Strain

Only alpha-amylase and pressure had a significant influence on the value of the yield stress and strain. Alpha-amylase had a quadratic effect on the yield stress whereas the pressure had a quadratic effect on the yield strain. Alpha-amylase and pressure had also a combined effect on the yield strain. The final equation and R-square values were:

Yield Stress =
$+0,09$
$-1,17*10^{-4}$ * Pressure
$-5,77*10^{-6}$ * Alpha-amylase
$+1,60*10^{-9}$ * Alpha-amylase^2

R-squared = 0.60

Yield Strain =
$+6110$
$-24,2$ *Pressure
$-0,25$ *Alpha-amylase
$+6,74*10^{-4}$ *Pressure*Alpha-amylase
$+0,03$ * Pressure^2

R-squared = 0.83

The yield stress rose when the amount of alpha-amylase increased, and it lowered slightly when the pressure grew. Both alpha-amylase and pressure had a decreasing effect on the yield strain.

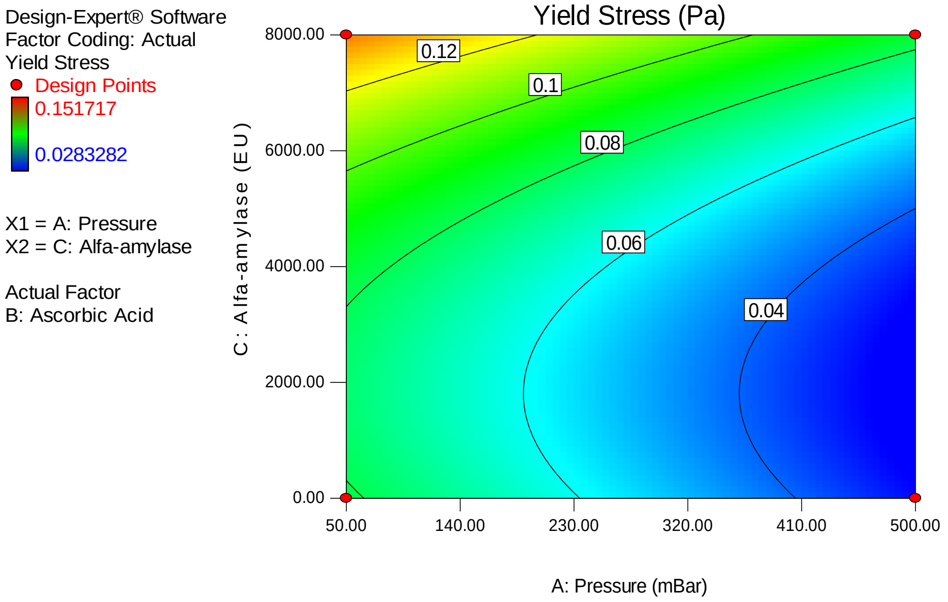


Figure 8. Contour image of yield stress.

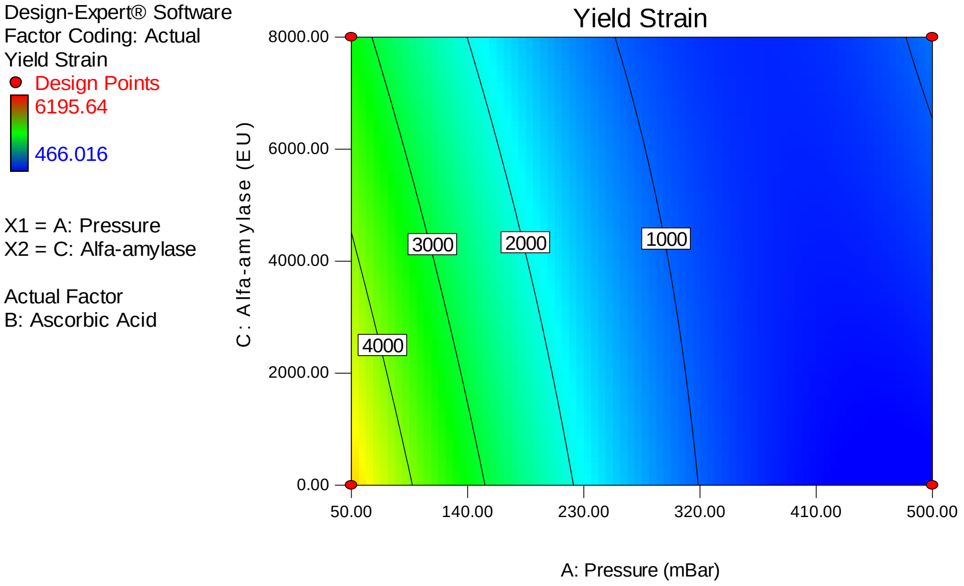


Figure 9. Contour image of yield strain.

3.2 Collapse Stress and Strain

All three variables had an influence on collapse stress and collapse strain. There were combined effects on the value of collapse stress with ascorbic acid and alpha-amylase. The pressure had a contrary combined effect on the collapse strain with ascorbic acid and alpha-amylase. The pressure had also a quadratic effect on the value of the collapse strain. The final equation and R-square values were:

Collapse Stress =
+0,37
$-1{,}07*10^{-4}$ *Pressure
$-3{,}39*10^{-4}$ *Ascorbic Acid
$-5{,}65*10^{-6}$ *Alpha-amylase
$+1{,}60*10^{-7}$ *Ascorbic Acid * Alpha-amylase

R-squared = 0.46

Collapse Strain =
+25200
-80,4 * Pressure
-71,9 * Ascorbic Acid
-1,19 * Alpha-amylase
+0,17 * Pressure * Ascorbic Acid
$+2{,}38*10^{-3}$ * Pressure * Alpha-amylase
+0,07 * Pressure^2

R-squared = 0.88

The ascorbic acid increased the value of collapse stress more than alpha-amylase. Under the influence of both, the collapse stress increased efficiently comparing to the effect of any individual factor. When examining the effect of pressure variances, increasing the amount of pressure decreased the value of the collapse stress steadily in each measurement point.

The more pressure, alpha-amylase or pressure attended, the lower was the value of the collapse strain. Ascorbic acid had not got a major impact on the collapse strain when the pressure was relatively high. The alpha-amylase had a stronger decreasing effect on the value comparing to ascorbic acid.

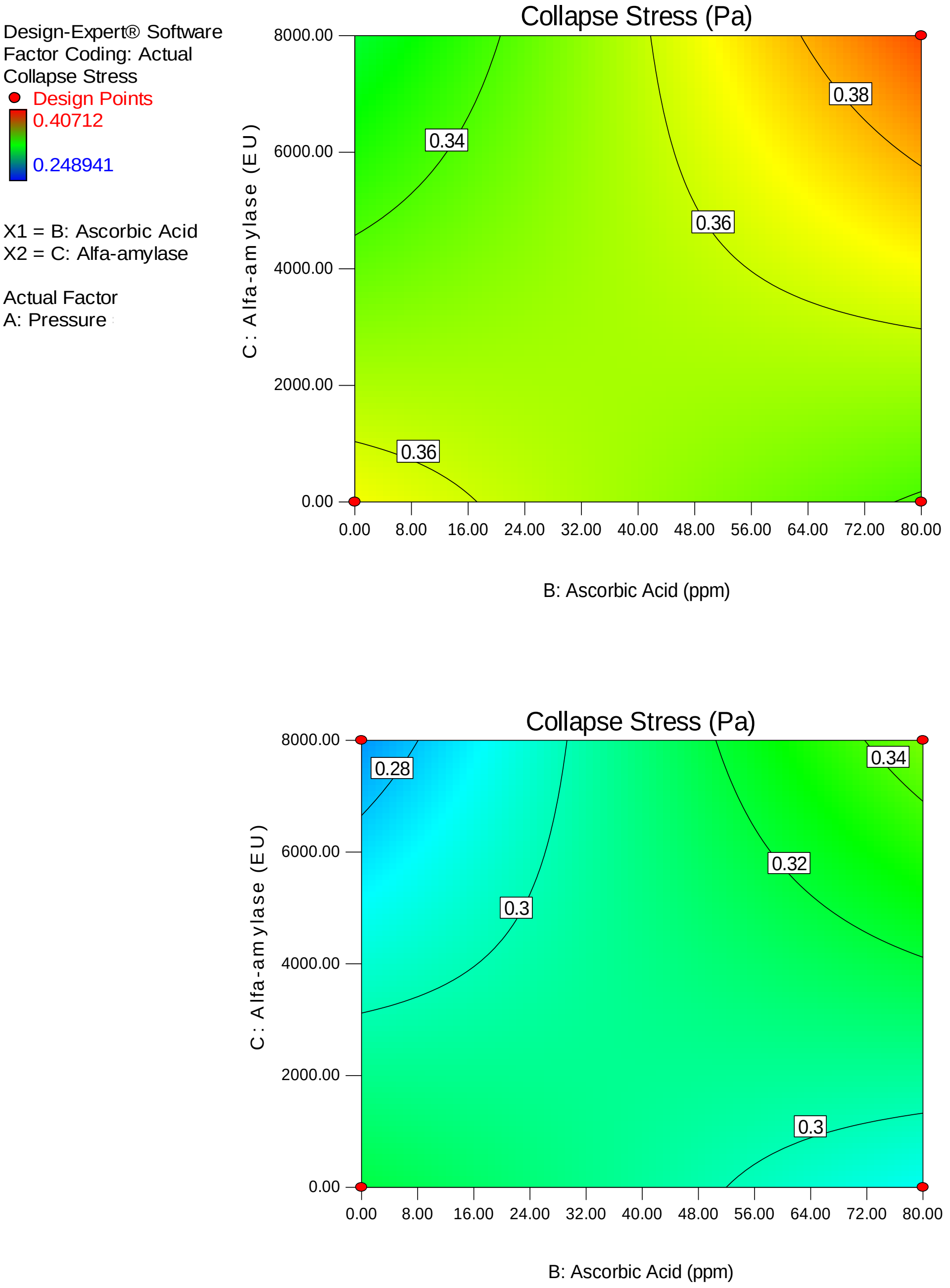


Figure 10. Contour image of collapse stress.

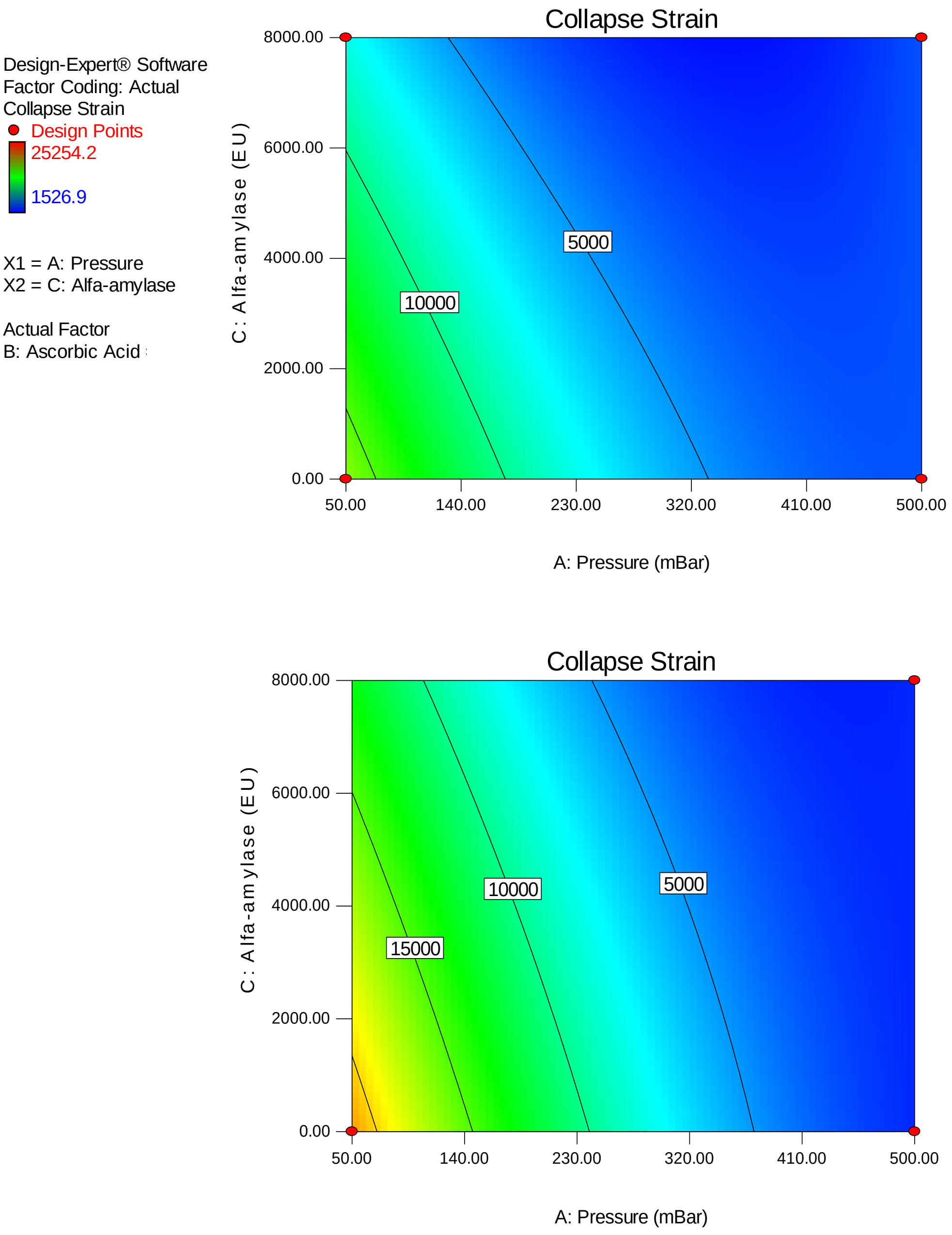


Figure 11. Contour image of collapse strain.

3.3 Elasticity of Crumb and Solid Phase

The amount of ascorbic acid did not have a significant effect on the elasticity of the crumb and the elasticity of the solid phase. The pressure and alpha-amylase had a combined effect on the elasticity of the crumb, and the pressure had also a quadratic effect. The pressure and alpha-amylase affected the elasticity of solid phase individually and linearly. The final equation and R-square values were:

Elasticity of crumb =

$$\begin{aligned} &+70400 \\ &-206 && * \text{Pressure} \\ &-5{,}35 && * \text{Alpha-amylase} \\ &+0{,}01 && * \text{Pressure} * \text{Alpha-amylase} \\ &+0{,}18 && * \text{Pressure}^2 \end{aligned}$$

R-squared = 0.88

Elasticity solid phase =

$$\begin{aligned} &+6{,}79*10^5 \\ &-267 && * \text{Pressure} \\ &-15{,}9 && * \text{Alpha-amylase} \end{aligned}$$

R-squared = 0.65

The elasticity of the crumb was the highest when there was no alpha-amylase and the pressure was very low. In the low pressure, increasing the amount of alpha-amylase dramatically decreased the elasticity of the crumb. When the pressure was 500mBar, the amount of alpha-amylase did not have much influence on the matter anymore.

The elasticity of the solid phase linearly rose, when the amount of alpha-amylase and pressure decreased. The highest value of elasticity was reached when the pressure was the lowest and when there was no alpha-amylase used.

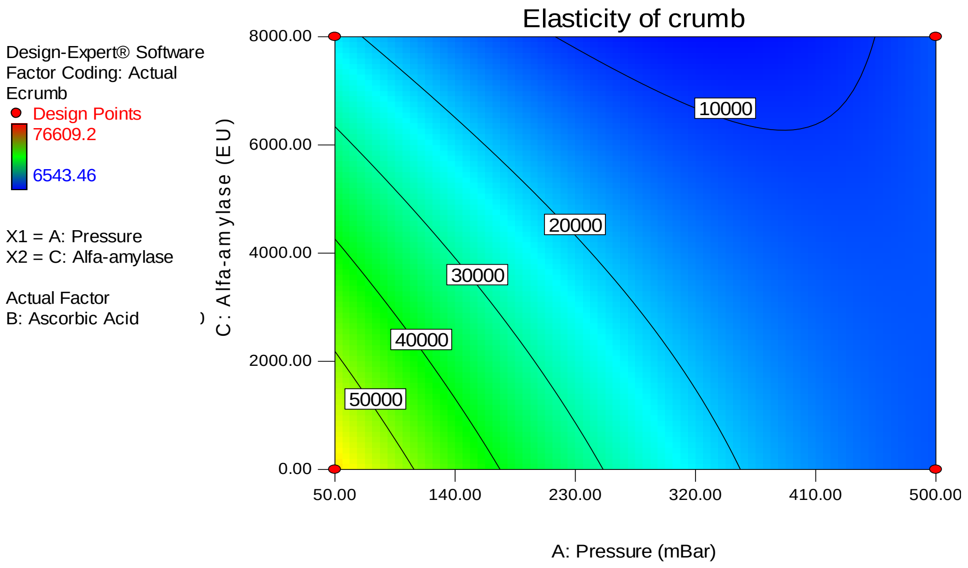


Figure 12. Contour image of elasticity of crumb.

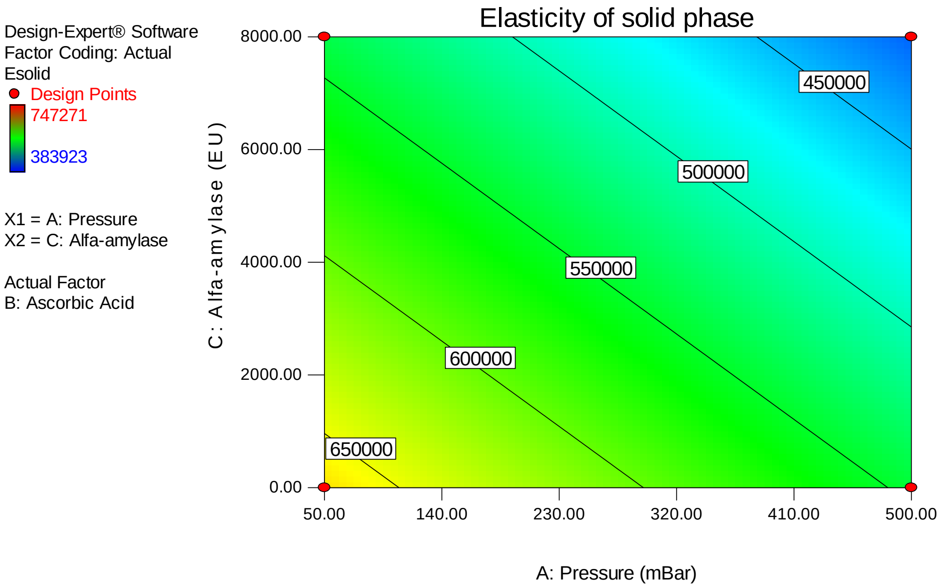


Figure 13. Contour image of elasticity of solid phase.

3.4 Storage and Loss Modulus

The variety of pressure did not have any influence on the storage modulus or loss modulus of the crumb. Only ascorbic acid and alpha-amylase both had an individual and linear effect on the values of storage and loss modulus. The final equation and R-square values were:

Storage Modulus =
$+1,21*10^6$
-3760 * Ascorbic Acid
+45,4 * Alpha-amylase

R-squared = 0.34

Loss Modulus =
$+3,61*10^5$
-1593 * Ascorbic Acid
+23,4 * Alpha-amylase

R-squared = 0.53

The amount of alpha-amylase had an increasing influence on the storage modulus value, but if ascorbic acid was also added, together they decreased the value. All in all, the value of the storage modulus did not change much despite the alpha-amylase and ascorbic acid. The minor change in the value of the storage modulus can be seen in the figure.

The effects of alpha-amylase and ascorbic acid worked quite the same way in the loss modulus as in the storage modulus. However, the effect of the increasing amount of alpha-amylase has more significance in this case.

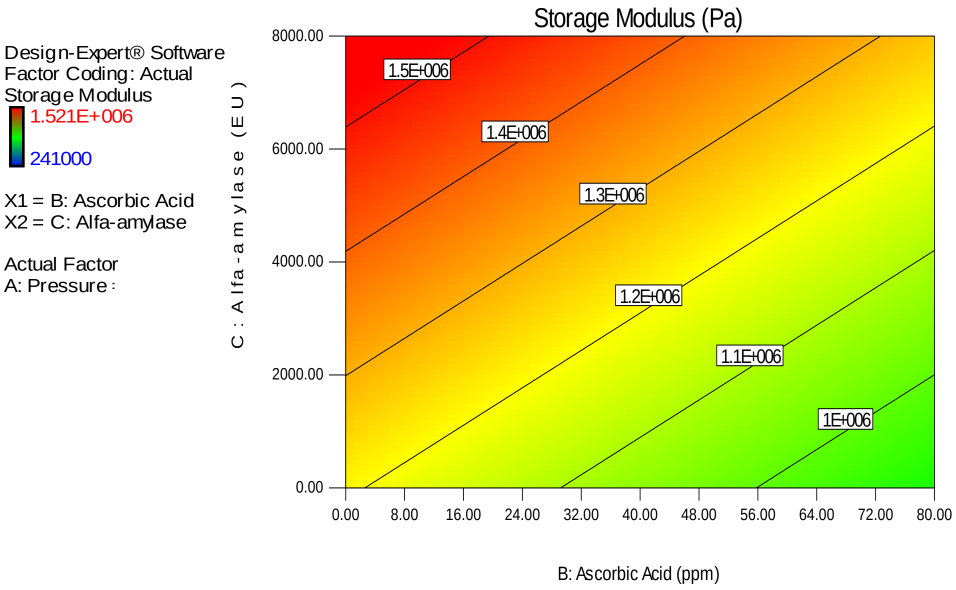


Figure 14. Contour image of storage modulus.

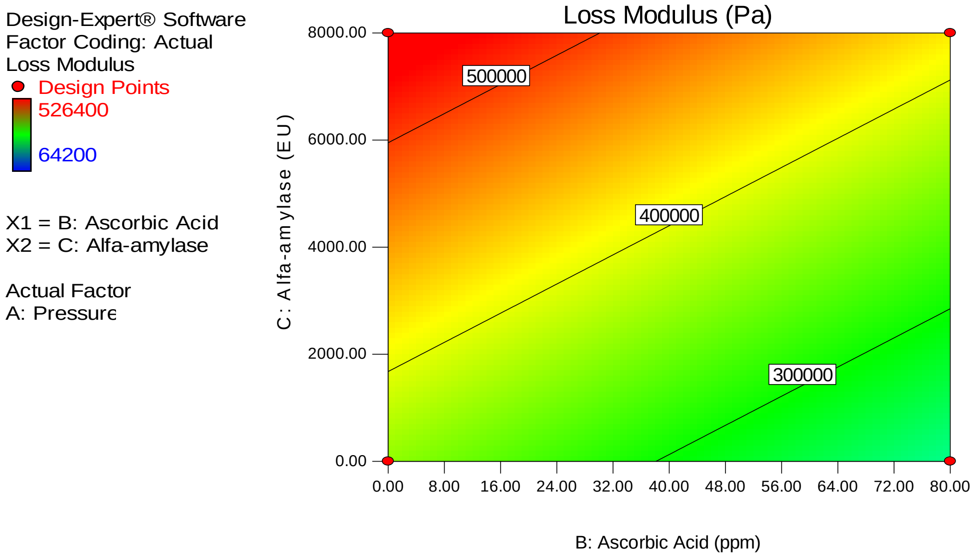


Figure 15. Contour image of loss modulus.

3.5 Image Analysis

Surface of slices

All three variables had a small influence on the surface of the slices. However, the ascorbic acid had also a quadratic influence. The final equation and R-square values were:

$$
\begin{array}{ll}
\text{Surface of Slices} = & \\
\quad +0{,}01 & \\
\quad +1{,}70 \cdot 10^{-5} & * \text{ Pressure} \\
\quad +1{,}60 \cdot 10^{-4} & * \text{ Ascorbic Acid} \\
\quad -4{,}31 \cdot 10^{-7} & * \text{ Alpha-amylase} \\
\quad -1{,}82 \cdot 10^{-6} & * \text{ Ascorbic Acid}^2
\end{array}
$$

R-squared = 0.59

With no ascorbic acid or with the highest amount of ascorbic acid, the surfaces of the slices were the smallest. Also alpha-amylase decreased the volume of the bread. The pressure had a most direct effect on the volume and, therefore, the surface of the slices increased along with pressure.

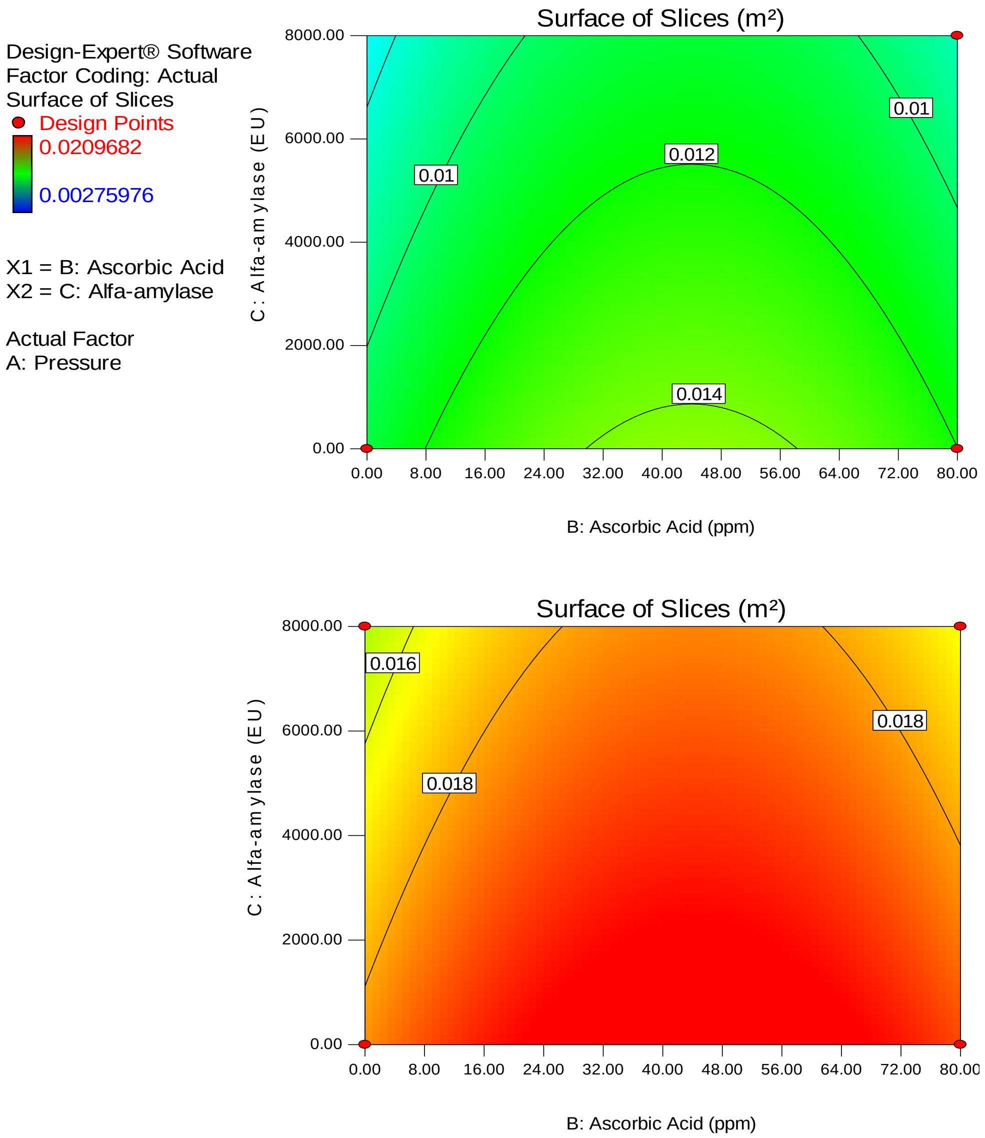


Figure 16. Contour image of surface of slices.

Surface of pores

Each of the three factors had not only an individual influence, but also a combined influence on the surface of the pores. The pressure and ascorbic acid had quadratic effect on the surface. The final equation and R-square values were:

Surface of Pores =
$+2{,}23 \cdot 10^{-4}$
$1{,}16 \cdot 10^{-6}$ * Pressure
$-2{,}94 \cdot 10^{-5}$ * Ascorbic Acid
$+2{,}67 \cdot 10^{-7}$ * Alpha-amylase
$-1{,}06 \cdot 10^{-7}$ * Pressure * Ascorbic Acid
$-1{,}43 \cdot 10^{-9}$ * Pressure * Alpha-amylase
$+7{,}54 \cdot 10^{-9}$ * Ascorbic Acid * Alpha-amylase
$+1{,}74 \cdot 10^{-8}$ * Pressure^2
$+5{,}02 \cdot 10^{-7}$ * Ascorbic Acid^2

R-squared = 0.85

The largest surface of the pores was reached when 8000 EU alpha amylase and 80 ppm ascorbic acid was used, and the mixing took place in 50 mBar. The combined influence of the factors reduced when the pressure was higher.

Ascorbic acid weakened the increasing effect of the pressure on the surface of the pores, without the presence of alpha-amylase. If the highest amount of alpha-amylase was used and no ascorbic acid at all, the pressure did not change the surface of the pores. The effect of alpha-amylase and ascorbic acid was quite the opposite in lower pressure than in higher. Nevertheless, the pressure had mainly an increasing effect on the surface of the pores.

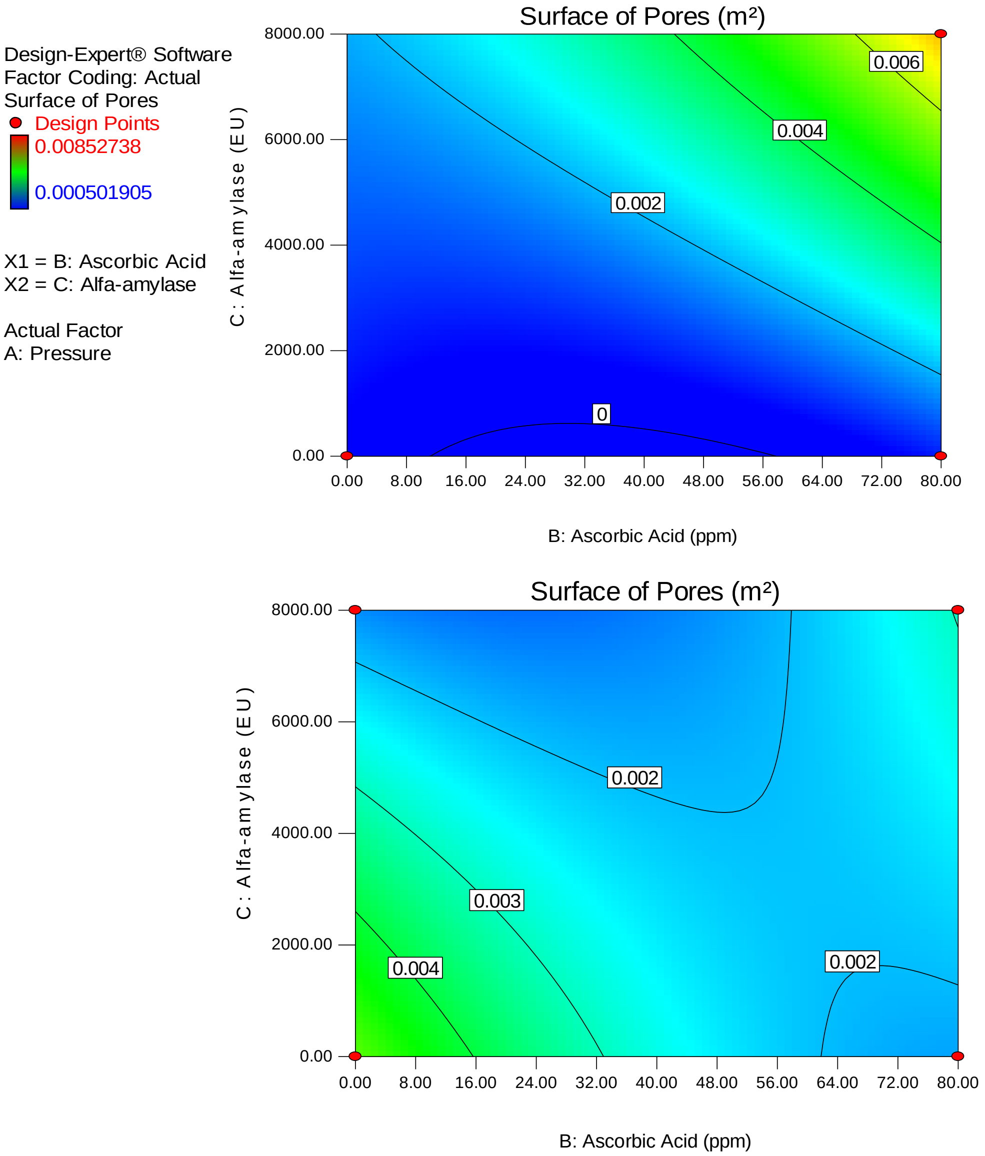


Figure 17. Contour image of surface of pores.

Numbers of pores

All three factors influenced the numbers of the pores, and they also had a combined effect. However, none of the factors had a quadratic influence. The final equation and R-square values were:

Numbers of Pores =
+5590
+45,3 * Pressure
+19,2 * Ascorbic Acid
+0,06 * Alpha-amylase
-0,21 * Pressure * Ascorbic Acid
$-3{,}84*10^{-3}$ * Pressure * Alpha-amylase
+0,03 * Ascorbic Acid * Alpha-amylase

R-squared = 0.80

Pressure mainly increased the number of the pores, especially when there was no alpha-amylase or ascorbic acid. Yet the value stayed the same, if the amount of ascorbic acid and alpha-amylase was highest. Ascorbic acid slightly increased the number of the pores.

Alpha-amylase and ascorbic acid had a considerable joint effect on the number of the pores. In 500mBar, pressure with the highest amount of alpha amylase, ascorbic acid increased the number of the pores slightly, and if there was no alpha-amylase the number decreased slightly. When the pressure was at its lowest and alpha-amylase at its highest, ascorbic acid had an increasing effect on the number of the pores.

Alpha-amylase did not have a significant importance when the pressure was low and there was no ascorbic acid used. However, if there was a high amount of ascorbic acid in those circumstances, alpha-amylase increased the number of pores. Alpha-amylase decreased the number, if the pressure was at its highest, and there was no ascorbic acid, but if there was a high amount of ascorbic acid, the number slightly increased. However, the pressure had an increasing effect on the number of the pores.

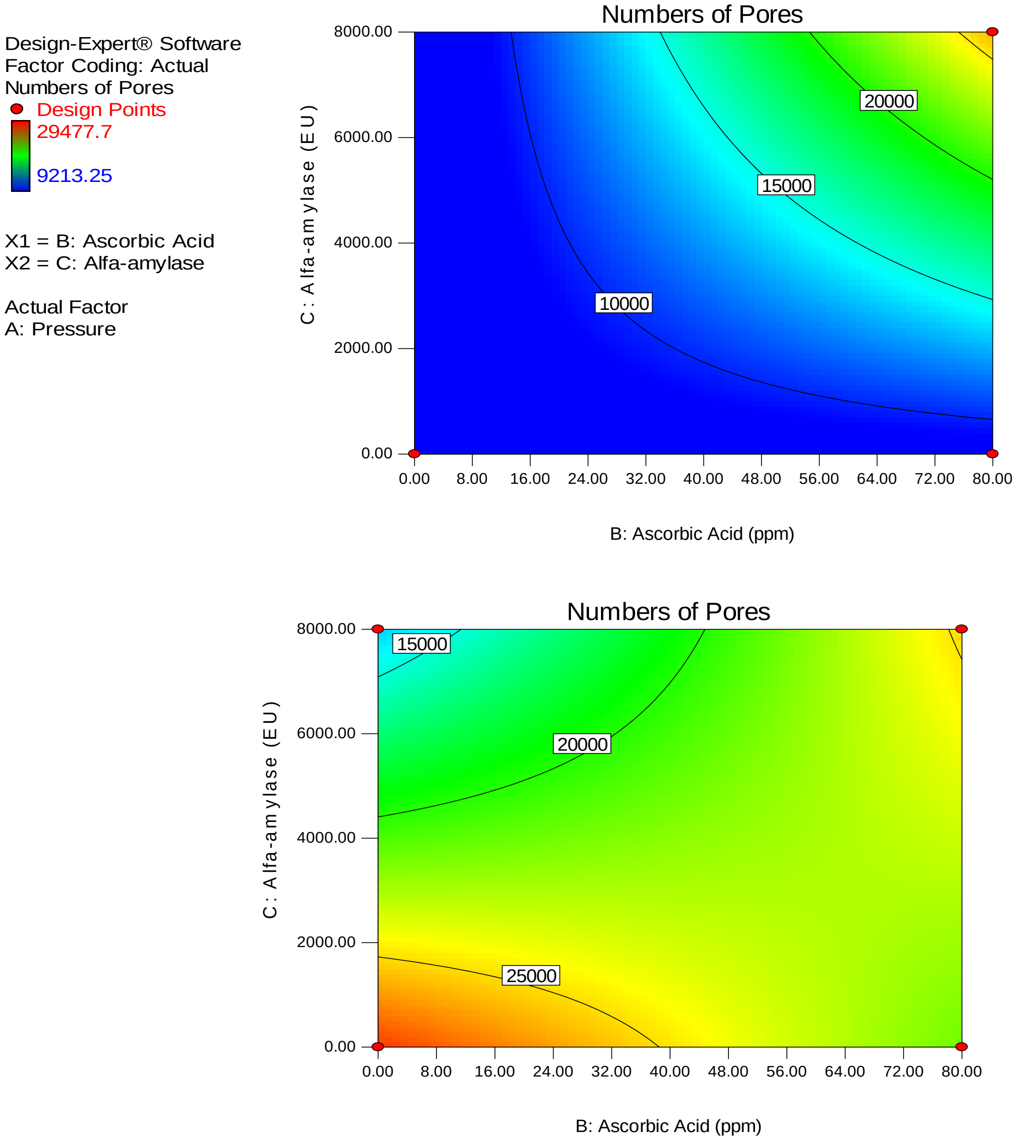


Figure 18. Contour picture of number of pores.

Apparent Porosity

The same factors affected apparent porosity as the numbers of the pores. The final equation and R-square values were:

Apparent Porosity =

$$
\begin{array}{ll}
-65{,}2 & \\
+0{,}28 & * \text{ Pressure}\\
+0{,}92 & * \text{ Ascorbic Acid}\\
+6{,}98*10^{-3} & * \text{ Alpha-amylase}\\
-4{,}90*10^{-3} & * \text{ Pressure } * \text{ Ascorbic Acid}\\
-4{,}19*10^{-5} & * \text{ Pressure } * \text{ Alpha-amylase}\\
+2{,}67*10^{-4} & * \text{ Ascorbic Acid } * \text{ Alpha-amylase}
\end{array}
$$

$R\text{-squared} = 0.62$

Pressure had an increasing influence on the apparent porosity, if there was no alpha-amylase or ascorbic acid. If there was one of the two, the pressure increased the value slightly and when both of the two factors had reached their maximum value, the apparent porosity decreased sharply.

Ascorbic acid slightly increased the value, when the pressure and alpha-amylase had reached their lowest values. In low pressure and with the maximum amount of alpha-amylase, ascorbic acid increased the porosity very effectively. If the pressure also was also high, the effect of ascorbic acid was increasing but not so dramatically. In low pressure and with no alpha-amylase, the value decreased.

Alpha-amylase did not make a lot of difference, if the pressure was 50 mBar and there was no ascorbic acid. The value increased sharply in circumstances when a 50mBar pressure and the maximum amount ascorbic acid were used. When the factors had the opposite value of 500 mBar pressure and no ascorbic acid, the porosity decreased. If both factors had maximum value, alpha-amylase had an increasing effect on the porosity.

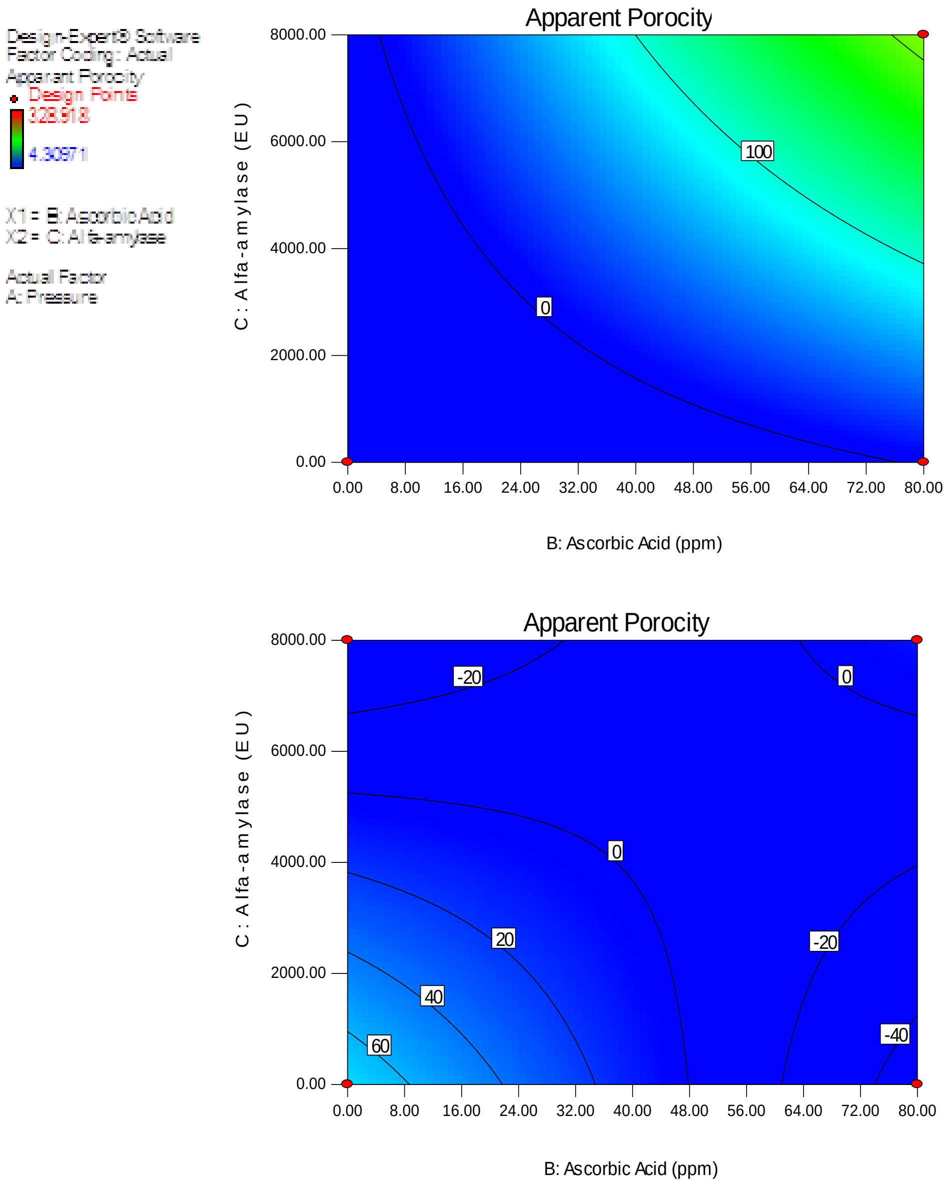


Figure 19. Contour image of apparent porosity.

Surface of Solid Phase

Ascorbic acid and alpha-amylase had a combined effect on the surface of solid phase. Nevertheless, pressure and ascorbic acid had a quadratic effect. Pressure had mainly an increasing effect on the surface. The final equation and R-square values were:

Surface of Solid phase =
$+0,01$
$+2,71*10^{-5}$ * Pressure
$+8,52*10^{-5}$ * Ascorbic Acid
$-8,14*10^{-7}$ * Alpha-amylase
$+1,30*10^{-8}$ * Ascorbic Acid * Alpha-amylase
$-3,01*10^{-8}$ * Pressure^2
$-1,26*10^{-6}$ * Ascorbic Acid^2

R-squared = 0.77

In any pressure, the value of the ascorbic acid mainly increased the surface of the solid phase if there was the maximum amount of alpha-amylase. If there was a low amount of alpha-amylase, the minimum surface of the solid phase was reached when there was no ascorbic acid at all or the maximum amount of it was used. Alpha-amylase decreased the surface if there was no ascorbic acid and increased it if there was a high amount of ascorbic acid.

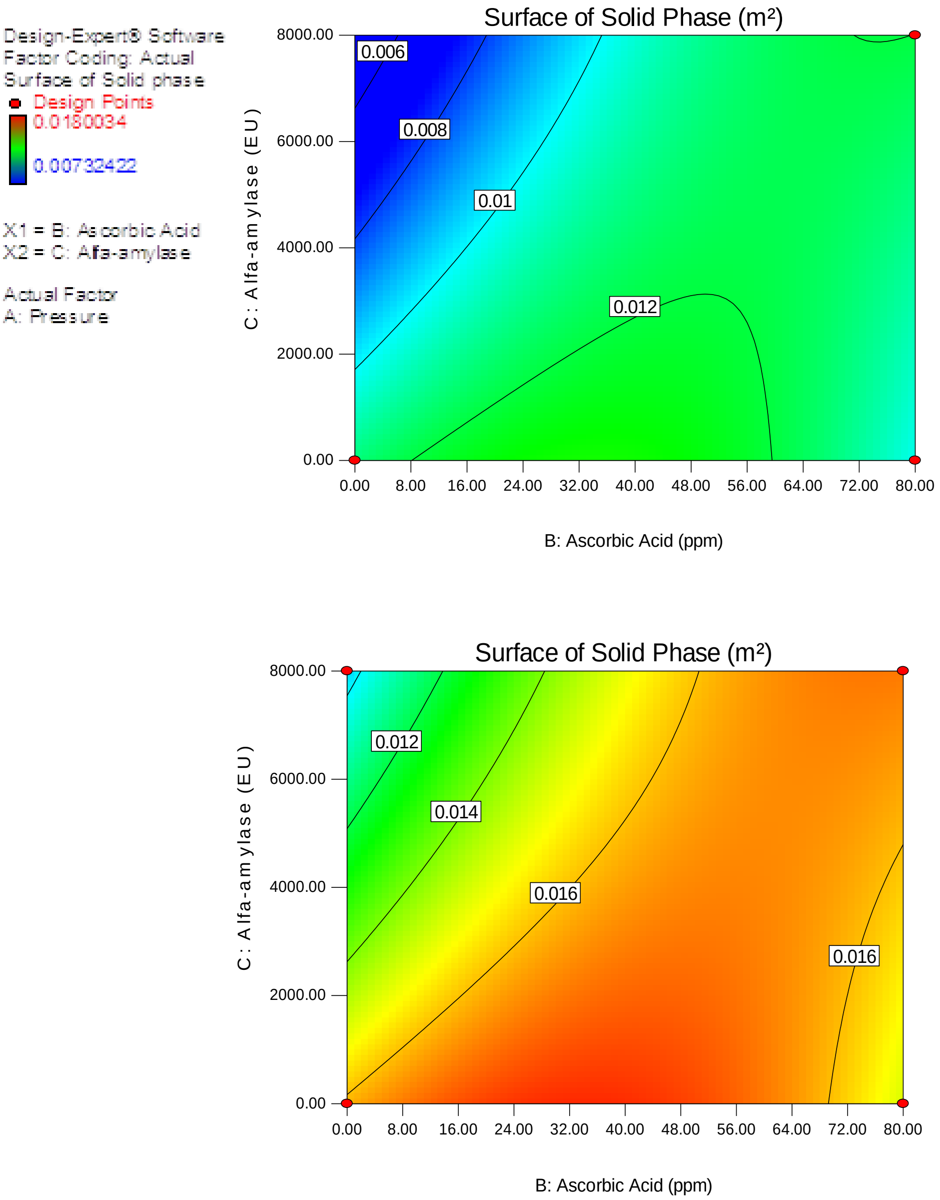


Figure 20. Contour image of surface of solid phase.

4 SUMMARY AND CONCLUSION

4.1 Conclusions

All the factors had a significant influence on some of the measurements. Because of the research layout, the effect of the factors may not be considered individually. Alpha-amylase had a significant influence on every feature. All three factors had an influence especially on the results of image analysis but also on the collapse stress and collapse strain. From the Table 1 it can be deduced that the porous structure of both, crumb and slices, are more sensitive to variation than other factors.

The letters in the Table 1 stand for:

A = pressure
B = ascorbic acid
C = alpha-amylase
AB = combined effect of pressure and ascorbic acid
AC = combined effect of pressure and alpha-amylase
BC = combined effect of ascorbic acid and alpha-amylase
A^2 = quadratic effect of pressure
B^2 = quadratic effect of ascorbic acid
C^2 = quadratic effect of alpha-amylase
R^2 = R-square

Table 1. Factors that had significant influence on results and R-square of the measured feature.

	A	B	C	AB	AC	BC	A^2	B^2	C^2	R^2
Yield Stress	x		x						x	60%
Yield Strain	x		x		x		x			83%
Collapse Stress	x	x	x			x				46%
Collapse Strain	x	x	x	x	x		x			88%
Elasticity of Crumb	x		x		x		x			89%
Elasticity of Solid Phase	x		x							65%
Storage Modulus		x	x							34%
Loss Modulus		x	x							53%
Surface of Slices	x	x	x					x		59%
Surface of Pores	x	x	x	x	x	x	x	x		85%
Numbers of Pores	x	x	x	x	x	x				80%
Apparent Porosity	x	x	x	x	x	x				62%
Surface of Solid Phase	x	x	x			x	x	x		77%
Total	**11**	**9**	**13**	**4**	**6**	**5**	**5**	**3**	**1**	

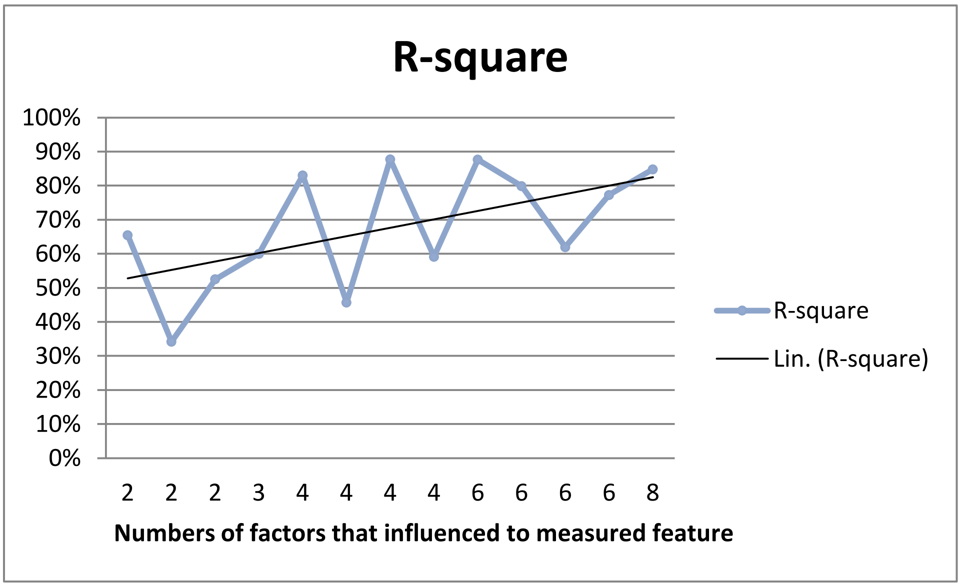


Figure 21. Correlation between numbers of influenced factors to value of R-square.

From Figure 21 a trend can be seen where the percentage of R-square rose along with the number of factors that had an influence on the features measured. The explanation was that if there were fewer factors influencing a feature, the more measurement points remained far from results. When the R-square was more than 80% the results fitted well to the line.

Alpha-amylase was predicted to have a negative effect on the pores and structure. In most results of the image analysis, that was the case. What comes to apparent porosity, in the lowest pressure used, alpha-amylase had a slightly increasing effect. The influence was quite the opposite if there was also ascorbic acid. However, the surface of the slices still decreased with the influence of alpha-amylase and ascorbic acid. Alpha-amylase did not improve the elasticity of the bread, and it increased the values of the storage and loss modulus. It had an influence on the viscoelastic behaviour of the crumb.

Mixing in vacuum was predicted to have a decreasing effect on the number of gas cells. The lower the pressure was, the smaller the number of pores was. The only values that the pressure did not have an influence on were the storage and loss modules. Therefore, using vacuum between 50-500 mBar had no influence on the refundable energy of the crumb or released energy under stress. Pressure did have an influence on the elasticity and solid phase of the crumb together with alpha-amylase. The value of the elasticity decreased when the pressure grew. The pressure did not affect the viscous properties of the crumb because it did not have an effect on the parts of dynamic modulus.

Ascorbic acid was predicted to increase the volume of bread. According to the results of this study ascorbic acid increased the surface of the slices to a certain point. If the concentration of ascorbic acid was too high, the volume started to decrease. In the minimum pressure, the surface of the slices started to decrease when there was over 45ppm of ascorbic acid. In the maximum pressure, the surface started to decrease when there was over 49ppm of ascorbic acid. The effect of ascorbic acid was not linear but quadratic. Ascorbic acid decreased the value of the storage and loss modulus but it did not have any influence on the elasticity of the bread.

4.2 Suggestion

The results can be used to improve certain rheological or structural quality of bread. If the elastic properties are needed, a lot of alpha-amylase should not be used. For instance, if the volume of the bread and the porosity should be improved, ascorbic acid should be used. Baking in vacuum lowers the elasticity of the crumb. The study also can be used for evaluating how human factor influences to the results.

For an accurate model the minimum and maximum values of the factors used should be optimized. For example, the amount of alpha-amylase used was too high when the dough was mixed in 50 mBar. The measurements were difficult to do and the bread was sticky. However, this study gives a good framework for that and shows a correlation between different features measured and factors that had an influence on them.

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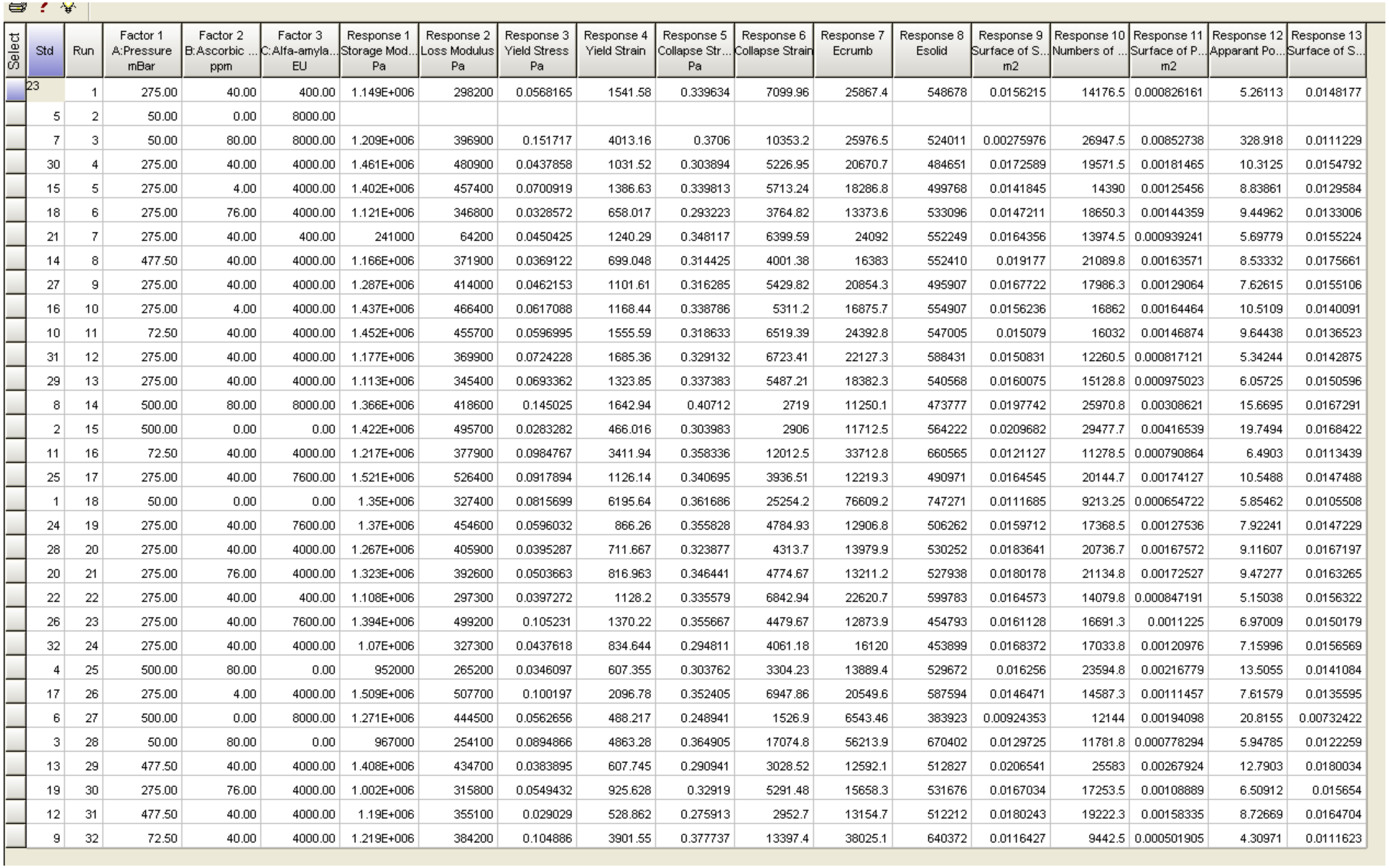
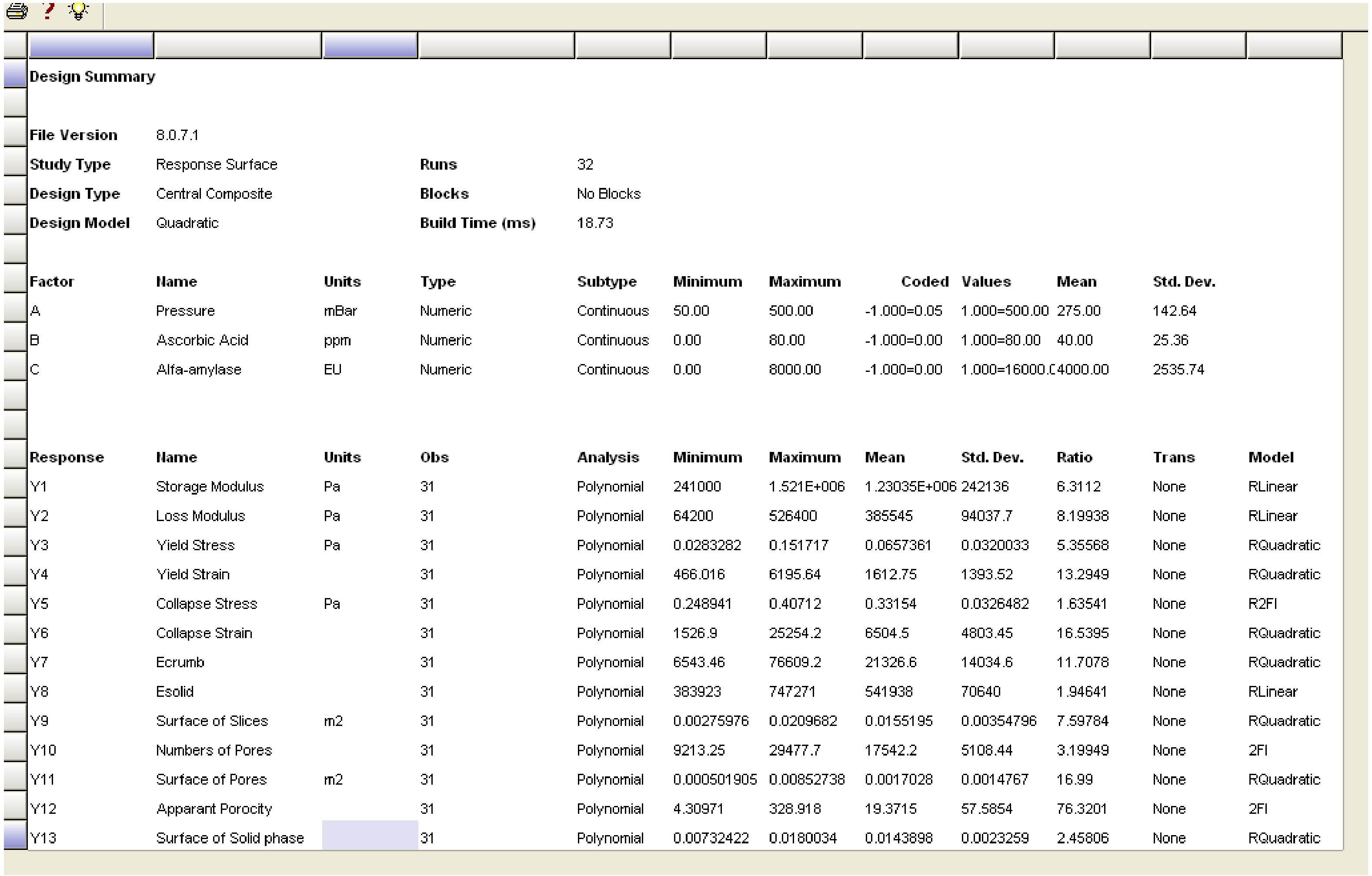
ATTACHMENTS

ATTACHMENT 1. Actual design and results.



Select	Std	Run	Factor 1 A:Pressure mBar	Factor 2 B:Ascorbic ... ppm	Factor 3 C:Alfa-amyla... EU	Response 1 Storage Mod... Pa	Response 2 Loss Modulus Pa	Response 3 Yield Stress Pa	Response 4 Yield Strain	Response 5 Collapse Str... Pa	Response 6 Collapse Strain	Response 7 Ecrumb	Response 8 Esolid	Response 9 Surface of S... m2	Response 10 Numbers of ...	Response 11 Surface of P... m2	Response 12 Apparant Po...	Response 13 Surface of S...
	23	1	275.00	40.00	400.00	1.149E+006	298200	0.0568165	1541.58	0.339634	7099.96	25867.4	548678	0.0156215	14176.5	0.000826161	5.26113	0.0148177
	5	2	50.00	0.00	8000.00													
	7	3	50.00	80.00	8000.00	1.209E+006	396900	0.151717	4013.16	0.3706	10353.2	25976.5	524011	0.00275976	26947.5	0.00852738	328.918	0.0111229
	30	4	275.00	40.00	4000.00	1.461E+006	480900	0.0437858	1031.52	0.303894	5226.95	20670.7	484651	0.0172589	19571.5	0.00181465	10.3125	0.0154792
	15	5	275.00	4.00	4000.00	1.402E+006	457400	0.0700919	1386.63	0.339813	5713.24	18286.8	499768	0.0141845	14390	0.00125456	8.83861	0.0129584
	18	6	275.00	76.00	4000.00	1.121E+006	346800	0.0328572	658.017	0.293223	3764.82	13373.6	533096	0.0147211	18650.3	0.00144359	9.44962	0.0133006
	21	7	275.00	40.00	400.00	241000	64200	0.0450425	1240.29	0.348117	6399.59	24092	552249	0.0164356	13974.5	0.000939241	5.69779	0.0155224
	14	8	477.50	40.00	4000.00	1.166E+006	371900	0.0369122	699.048	0.314425	4001.38	16383	552410	0.019177	21089.8	0.00163571	8.53332	0.0175661
	27	9	275.00	40.00	4000.00	1.287E+006	414000	0.0462153	1101.61	0.316285	5429.82	20854.3	495907	0.0167722	17986.3	0.00129064	7.62615	0.0155106
	16	10	275.00	4.00	4000.00	1.437E+006	466400	0.0617088	1168.44	0.338786	5311.2	16875.7	554907	0.0156236	16862	0.00164464	10.5109	0.0140091
	10	11	72.50	40.00	4000.00	1.452E+006	455700	0.0596995	1555.59	0.318633	6519.39	24392.8	547005	0.015079	16032	0.00146874	9.64438	0.0136523
	31	12	275.00	40.00	4000.00	1.177E+006	369900	0.0724228	1685.36	0.329132	6723.41	22127.3	588431	0.0150831	12260.5	0.000817121	5.34244	0.0142875
	29	13	275.00	40.00	4000.00	1.113E+006	345400	0.0693362	1323.85	0.337383	5487.21	18382.3	540568	0.0160075	15128.8	0.000975023	6.05725	0.0150596
	8	14	500.00	80.00	8000.00	1.366E+006	418600	0.145025	1642.94	0.40712	2719	11250.1	473777	0.0197742	25970.8	0.00308621	15.6695	0.0167291
	2	15	500.00	0.00	0.00	1.422E+006	495700	0.0283282	466.016	0.303983	2906	11712.5	564222	0.0209682	29477.7	0.00416539	19.7494	0.0168422
	11	16	72.50	40.00	4000.00	1.217E+006	377900	0.0984767	3411.94	0.358336	12012.5	33712.8	660565	0.0121127	11278.5	0.000790864	6.4903	0.0113439
	25	17	275.00	40.00	7600.00	1.521E+006	526400	0.0917894	1126.14	0.340695	3936.51	12219.3	490971	0.0164545	20144.7	0.00174127	10.5488	0.0147488
	1	18	50.00	0.00	0.00	1.35E+006	327400	0.0815699	6195.64	0.361686	25254.2	76609.2	747271	0.0111685	9213.25	0.000654722	5.85462	0.0105508
	24	19	275.00	40.00	7600.00	1.37E+006	454600	0.0596032	866.26	0.355828	4784.93	12906.8	506262	0.0159712	17368.5	0.00127536	7.92241	0.0147229
	28	20	275.00	40.00	4000.00	1.267E+006	405900	0.0395287	711.667	0.323877	4313.7	13979.9	530252	0.0183641	20736.7	0.00167572	9.11607	0.0167197
	20	21	275.00	76.00	4000.00	1.323E+006	392600	0.0503663	816.963	0.346441	4774.67	13211.2	527938	0.0180178	21134.8	0.00172527	9.47277	0.0163265
	22	22	275.00	40.00	400.00	1.108E+006	297300	0.0397272	1128.2	0.335579	6842.94	22620.7	599783	0.0164573	14079.8	0.000847191	5.15038	0.0156322
	26	23	275.00	40.00	7600.00	1.394E+006	499200	0.105231	1370.22	0.355667	4479.67	12873.9	454793	0.0161128	16691.3	0.0011225	6.97009	0.0150179
	32	24	275.00	40.00	4000.00	1.07E+006	327300	0.0437618	834.644	0.294811	4061.18	16120	453899	0.0168372	17033.8	0.00120976	7.15996	0.0156569
	4	25	500.00	80.00	0.00	952000	265200	0.0346097	607.355	0.303762	3304.23	13889.4	529672	0.016256	23594.8	0.00216779	13.5055	0.0141084
	17	26	275.00	4.00	4000.00	1.509E+006	507700	0.100197	2096.78	0.352405	6947.86	20549.6	587594	0.0146471	14587.3	0.00111457	7.61579	0.0135595
	6	27	500.00	0.00	8000.00	1.271E+006	444500	0.0562656	488.217	0.248941	1526.9	6543.46	383923	0.00924353	12144	0.00194098	20.8155	0.00732422
	3	28	50.00	80.00	0.00	967000	254100	0.0894866	4863.28	0.364905	17074.8	56213.9	670402	0.0129725	11781.8	0.000778294	5.94785	0.0122259
	13	29	477.50	40.00	4000.00	1.408E+006	434700	0.0383895	607.745	0.290941	3028.52	12592.1	512827	0.0206541	25583	0.00267924	12.7903	0.0180034
	19	30	275.00	76.00	4000.00	1.002E+006	315800	0.0549432	925.628	0.32919	5291.48	15658.3	531676	0.0167034	17253.5	0.00108889	6.50912	0.015654
	12	31	477.50	40.00	4000.00	1.19E+006	355100	0.029029	528.862	0.275913	2952.7	13154.7	512212	0.0180243	19222.3	0.00158335	8.72669	0.0164704
	9	32	72.50	40.00	4000.00	1.219E+006	384200	0.104886	3901.55	0.377737	13397.4	38025.1	640372	0.0116427	9442.5	0.000501905	4.30971	0.0111623

ATTACHMENT 2. Design summary.



Design Summary

File Version	8.0.7.1		
Study Type	Response Surface	**Runs**	32
Design Type	Central Composite	**Blocks**	No Blocks
Design Model	Quadratic	**Build Time (ms)**	18.73

Factor	**Name**	**Units**	**Type**	**Subtype**	**Minimum**	**Maximum**	**Coded**	**Values**	**Mean**	**Std. Dev.**
A	Pressure	mBar	Numeric	Continuous	50.00	500.00	-1.000=0.05	1.000=500.00	275.00	142.64
B	Ascorbic Acid	ppm	Numeric	Continuous	0.00	80.00	-1.000=0.00	1.000=80.00	40.00	25.36
C	Alfa-amylase	EU	Numeric	Continuous	0.00	8000.00	-1.000=0.00	1.000=16000.	4000.00	2535.74

Response	**Name**	**Units**	**Obs**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
Y1	Storage Modulus	Pa	31	Polynomial	241000	1.521E+006	1.23035E+006	242136	6.3112	None	RLinear
Y2	Loss Modulus	Pa	31	Polynomial	64200	526400	385545	94037.7	8.19938	None	RLinear
Y3	Yield Stress	Pa	31	Polynomial	0.0283282	0.151717	0.0657361	0.0320033	5.35568	None	RQuadratic
Y4	Yield Strain		31	Polynomial	466.016	6195.64	1612.75	1393.52	13.2949	None	RQuadratic
Y5	Collapse Stress	Pa	31	Polynomial	0.248941	0.40712	0.33154	0.0326482	1.63541	None	R2FI
Y6	Collapse Strain		31	Polynomial	1526.9	25254.2	6504.5	4803.45	16.5395	None	RQuadratic
Y7	Ecrumb		31	Polynomial	6543.46	76609.2	21326.6	14034.6	11.7078	None	RQuadratic
Y8	Esolid		31	Polynomial	383923	747271	541938	70640	1.94641	None	RLinear
Y9	Surface of Slices	m2	31	Polynomial	0.00275976	0.0209682	0.0155195	0.00354796	7.59784	None	RQuadratic
Y10	Numbers of Pores		31	Polynomial	9213.25	29477.7	17542.2	5108.44	3.19949	None	2FI
Y11	Surface of Pores	m2	31	Polynomial	0.000501905	0.00852738	0.0017028	0.0014767	16.99	None	RQuadratic
Y12	Apparant Porocity		31	Polynomial	4.30971	328.918	19.3715	57.5854	76.3201	None	2FI
Y13	Surface of Solid phase		31	Polynomial	0.00732422	0.0180034	0.0143898	0.0023259	2.45806	None	RQuadratic

ATTACHMENT 3. 16-STD example picture from compression test results after analyzing in MATLAB.