MODELLING ALLOWABLE STORAGE TIME OF WHEAT AT 17% MOISTURE CONTENT

ΒY

EVELINE SCHROTH

A Thesis Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Biosystems Engineering University of Manitoba Winnipeg, Manitoba

© November, 1996



National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street Ottawa, Ontario K1A 0N4 Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington Ottawa (Ontario) K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan. distribute sell copies or of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à disposition la des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission. L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-612-16272-9

Canadä

EVELINE SCHROTH Name

Dissertation Abstracts International and Masters Abstracts International are arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation or thesis. Enter the corresponding four-digit code in the spaces provided.

SUBJECT TERM

AGRICULTURAL ENGINEERING

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture	0729
Art History	0377
Cinema	0900
Dance	0378
Design and Decorative Arts	0389
Fine Arts	0357
Information Science	0723
Journalism	0391
Landscape Architecture	0390
Library Ścience	0399
Mass Communications	0708
Music	0413
Speech Communication	0459
Thomton	0446

EDUCATION

General	.0515
Administration	0514
Adult and Continuina	0516
Agricultural	0517
Δ 	0273
Bilingual and Multicultural	0282
Business	0688
Community College	0275
Curriculum and Instruction	0727
Early Childhood	0519
Elementany	0510
Educational Psychology	0524
Eta a a a a a a a a a a a a a a a a a a	.0323
	.02//
Guidance and Counseling	.0519
rieglin	0680
Higher	.0745
History of	.0520
Home Economics	.0278
Industrial	.0521
Language and Literature	.0279
Mathematics	0280
Music	0522
Philosophy of	0998

Physical . Reading0535 Religious0527 Secondary 0533 Sociology of0340

LANGUAGE, LITERATURE AND LI

LINGUISTICS	
Language	
General	0679
Ancient	0289
Linguistics	0290
Modern	0291
Rhetoric and Composition	0681
Literature	
General	0401
Classical	0294
Comparative	0295
Medieval	0297
Modern	0298
African	0316
American	0591
Asian	0305
Canadian (English)	0352
Canadian (French)	0355
Caribbean	0360
English	0593
Germanic	0311
Latin American	0312
Middle Eastern	0315
Romance	0313
Slavic and East European	0314

.0370

THE SCIENCES AND ENGINEERING

Geodesy

BIOLOGICAL SCIENCES

B

B

E

Agriculture	
General	0473
Agronomy	0285
Animal Culture and	
Nutrition	0475
Animal Pathology	0476
Fisheries and Aquaculture	0792
Food Science and	_
Technology	0359
Forestry and Wildlife	0478
Plant Culture	0479
Plant Pathology	0480
Range Management	0777
Soil Science	0481
Wood Technology	0746
iology	
Géneral	0306
Anatomy	0287
Animal Physiology	0433
Biostatistics	0308
Botany	0309
Cell	0379
Ecology	0329
Entomology	0353
Genetics	0369
Limnology	0793
Microbiology	0410
Molecular	0307
Neuroscience	0317
Oceanography	0416
Plant Physiology	0817
Veterinary Science	0778
Zoology	0472
iophysics (
'Géneral	0786
Medical	0760
ARTH SCIENCES	
and the second	0 10 5

Biogeoc	hemis	hry	 	 	0423
Geocher	nistry		 	 	0996

Geology 0372 Geophysics 0373 Hydrology 0388 Mineralogy..... .0411 Paleobotany Paleoecology 0345 0426 **HEALTH AND ENVIRONMENTAL** SCIENCES

ain sciences	
General	.056
Audiology	0300
Donhetny	0547
Education y	
Education	.0350
Administration, Health Care	.0769
Human Development	0758
Immunology	098
Medicine and Surgery	056
Montal Unally	.000
	.034/
Nursing	.0565
Nutrition	.0570
Obstetrics and Gynecology.	.0380
Occupational Health and	
Salah	035
Oncology	.0992
Ophthalmology	.0381
Pathology	.0571
Pharmacology	.0415
Pharmacy	057
Public Hoalth	067
Dudiala an	.05/3
Kaalology	.05/4
Recreation	.0575
Rehabilitation and Therapy	.0382



PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy	0422
Religion	
General	0318
Biblical Studies	
Clergy	
History of	0320
Philosophy of	
Theology	0469

.....

SOCIAL SCI	ENCES	
American S	tudies	0323
Anthropolo		
Archa	Y	0224
Conoral	••••••	0320
mysical	•••••••	032/
Business Ad	ministration	
General		0310
Account	ina	0272
Banking		0770
Manaaa	moni	0454
Markali		0220
C High C	9,	
Cauagián 2		0385
Economics		
General		0501
Agricult	ural	0503
Commer	ce-Business	0505
Finance		0508
History		0500
labor.	************	0510
	••••••••••••••••	
ineory.		
rondore		.0358
Geography		.0358
Geography Gerontology	/	0358 0366 0351
Geography Gerontology History	/	0358 0366 0351
Geography Gerontology History General	/	0358 0366 0351
Geography Gerontology History General	······	0358 0366 0351 0578

Medieval	058	1
Modern	0.58	5
Church	033	ĉ
Black	032	ě
African	033	1
Asia, Australia and Oceania	033	Ż
Canadian	033	2
European	033	4
Latin American	033	ž
Middle Eastern	033	3
United States	033	ž
listory of Science	058	4
dw	039	ā
olitical Science		
General	061	5
International Law and		
Relations	061	6
Public Administration	061	7
Recreation	081	4
iocial Work	.045	2
ociology		
General	.062	6
Criminology and Penology	.062	7
Demography	.093	в
Ethnic and Racial Studies	.063	ì
Individual and Family		
Studies	.0628	B
Industrial and Labor		
Relations	.0629	9
Public and Social Welfare	.0630	Э
Social Structure and		
_ Development	0700	0
Theory and Methods	.034	4
ransportation	.0709	2
Irban and Regional Planning	.0999	7
Managada Chudian	A 461	ъ.

Speech Pathology	0460
Toxicology	0383
Home Economics	0386
PHISICAL SCIENCES	
Pure Sciences	
Chemistry	
General	0485
Agricultural	0749
Analytical	0486
Biochemistry	0487
Inorganic	0488
Nuclear	0739
Organic	0/00
Pharmacoutical	0470
Physical	0404
Pol mon	
Dedition	
Mathematics	
Manemancs	0405
rnysics	0/05
General	
Acoustics	0986
Astronomy and	
Astrophysics	0606
Almospheric Science	0608
Alomic	0748
Condensed Matter	0611
Electricity and Magnetism	0607
Elementary Particles and	
High Energy	0798
Fluid and Plasma	0759

Radiation0756 Statistics

Oplics

Applied Sciences

.0610

0463

care de badatas

Engineering General Aerospace Agricultural Automotive Biomedical Chemical0542 Civil Electronics and Electrical Environmental0775 Industrial Marine and Ocean Materials Science Mechanical Metallurgy Mining..... Nuclear Packaging Petroleum Sanitary and Municipal

Petroleum	0765
Sanitary and Municipal	.0554
System Science	0790
Geotechnology	.0428
Operations Research	0796
Plastics Technology	0795
Textile Technology	0994

0537

0538

0539

0541

.0543

0544

0546

0794

0548

0743

0551

0552

PSYCHOLOGY

CHARGE UI	
Behavioral	
Clinical	
Cognitive	
Developmental	.062
Experimental	062
Industrial	
Personality	062
Physiological	
Psýchobiology	
Psychometrics	
Social	

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

MODELLING ALLOWABLE STORAGE TIME OF WHEAT

AT 17% MOISTURE CONTENT

BY

EVELINE SCHROTH

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Eveline Schroth © 1996

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis/practicum, to the NATIONAL LIBRARY OF CANADA to microfilm this thesis/practicum and to lend or sell copies of the film, and to UNIVERSITY MICROFILMS INC. to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

ABSTRACT

The main objective in this study was to model the deterioration of wheat in storage. Practical limitations were required for the experiment, the first of which concerned the storage condition to be studied. The conditions studied were: 'Katepwa' wheat stored for 3 mo at 17% moisture content with temperatures ranging from 15 to 35°C. A second limitation was the definition of deterioration to be applied. Deterioration was defined by a decrease in germination to 90%.

Equations were derived to model the change in germination over time at 17% moisture content and five constant temperature conditions: 15, 20, 25, 30, and 35°C. The intention was to apply these equations in sequence to simulate decreasing temperature conditions. The experimental data, however, showed deterioration greater than that predicted by the equations. Evidently the equations do not account for some unknown aspect of the transformations occurring to the wheat while it is stored in a changing temperature environment.

An equation was derived to model the allowable storage time of fresh grain at 17% moisture content and at constant temperatures between 15 and 35°C. This equation is also applicable to changing temperature conditions when a deterioration index of 1.4 is assumed.

A decrease in germination to 90% preceded the first appearance of visible mould by 4-5 days. This decrease in germination was also correlated with a 25% increase in the fat acidity value of the wheat.

Further research is required to determine how the deterioration of wheat is affected by different moisture contents and different cultivars of wheat. As well, the changes that occur to wheat when the temperature is decreased during storage, require further study.

ii

ACKNOWLEDGEMENTS

I am grateful to my advisor, Dr. W.E. Muir, for his guidance and patience throughout my studies. As well, I am grateful to my committee members Dr. J.T. Mills, Dr. N.D.G. White, Dr. D. Abramson, and Dr. D.S. Jayas.

I thank Mr. M. McDonald, Mr. C. Demianyk, and Mr. M. Barron for the considerable technical assistance they provided.

I also thank the Natural Sciences and Engineering Research Council of Canada for the financial support of this project.

Finally, I am grateful to my husband David for his constant encouragement and support throughout this study, without which I would not have completed it.

TABLE OF CONTENTS

ABSTRACT
ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS iv
LIST OF FIGURES
LIST OF TABLES
1. INTRODUCTION 1 1.1 The need for a wheat deterioration equation 1 1.2 Objectives 3 1.3 Assumptions inherent to the study 3
2. LITERATURE REVIEW 4 2.1 Historical approaches to measuring grain deterioration 4 2.1.1 Deterioration parameters 4 2.1.2 Modelling deterioration of corn 6 2.1.3 Modelling deterioration of wheat 10 2.2 Stored grain deterioration parameters in this study 12
3. MATERIALS AND METHODS 15 3.1 Experimental setup 15 3.1.1 Experimental design 15 3.1.2 Experimental apparatus 18 3.1.3 Sampling frequency 20 3.2 Laboratory analysis of data 21 3.2.1 Preliminary condition of sample 21 3.2.2 Germination 21 3.2.3 Microflora identification 21 3.2.4 Visible mould detection 22 3.2.3 Free fatty acids detection 22
4. RESULTS 24 4.1 Rate of reduction in the germination over time 24 4.2 Allowable storage time equation 26 4.2.1 Constant conditions 26 4.2.2 Comparison of first and second sets of experiments 28 4.2.3 Decreasing temperature conditions 31 4.3 Correlation of other deterioration indices to loss in germination 35 4.3.1 Correlation of microflora activity to decrease in germination 35 4.3.2 Correlation of an increase in the fat acidity value with a decrease in germination 37
5. DISCUSSION

2

iv

5. 5. 5.	5.2 Allowable storage time	43 43 45 50 51
6. CONCI	LUSIONS	53
7. RECO	MMENDATIONS FOR FUTURE RESEARCH	55
8. REFER	RENCES	56
APPEND	IX A: Germination data	31
APPENDI	IX B: Visible mould data	35
APPENDI	IX C: Microflora data using the salted filter paper method	38
APPENDI	IX D: Microflora data using the filter paper method	3 3
APPENDI	IX E: Fat acidity value data	18
APPENDI	IX F: Moisture content data	21

.

v

LIST OF FIGURES

Figure 3.1.	Experimental chamber with apparatus
Figure 3.2.	Individual test containers holding grain bulks
Figure 4.1.	Changing germination of wheat at 17% m.c. and five constant temperature conditions
Figure 4.2.	Allowable storage times of wheat at 17% m.c
Figure 4.3.	Comparison of deterioration of samples in set 1 and set 2 with a similar temperature schedule
Figure 4.4	Comparison of predicted and measured allowable storage times for tests with changing temperatures
Figure 4.5.	Experimental and predicted germination under decreasing temperature conditions
Figure 4.6.	Allowable storage time as defined by germination drop compared with the first sign of visible mould
Figure 4.7.	Microflora examples with visible mould appearance and germination drop to 90%
Figure 4.8.	Change in the fat acidity value at constant conditions
Figure 4.9.	Correlation of the allowable storage time as defined by a 25% increase in FAV to the allowable storage time as defined by a decrease to 90% germination
Figure 5.1.	Deterioration curves for various crops in storage signifying that germination change is commonly sigmoidal
Figure 5.2.	Allowable storage time of wheat from Wallace et al. (1983) at two sets of moisture contents, with prediction Eq. (4.2b) (Section 4.2)
Figure 5.3.	Allowable storage time of wheat from Trisvyatskii (1969) and Mills (1992) with prediction Eq. (4.2b) (Section 4.2)
Figure 5.4.	Germination from the start of storage to the 90% germination cut off for allowable storage time
Figure 5.5.	Tests with similar temperature schedules at varying storage times 48

LIST OF TABLES

Table 3.1.	Temperature schedule
Table 4.1.	Storage temperature coefficients for Eq. (4.1)
Table 4.2.	Tests used to determine storage time at 35°C
Table 4.3.	Mean moisture content of each test
Table 4.4.	Time of the initial onset of deterioration as defined by a decrease in germination and the appearance of visible mould
Table 5.1.	Varying crop coefficients for Eq. (4.1) (Section 4.1)
Table 5.2.	Storage time of wheat roughly halved for every increase in temperature by 5°C
Table 5.3.	Length of storage at a given temperature after which cooling is still effective in delaying deterioration
Table 5.4.	Correlation coefficients for microflora, germination, and FAV

Vii

1. INTRODUCTION

1.1 The need for a wheat deterioration equation

Long-term storage of wheat in Canada is necessary to serve three basic functions:

1) to provide wheat throughout the year following harvest,

2) to provide wheat to the domestic grain processing industry and the animal feed industry, and

3) to provide wheat for export.

Spoilage of wheat can occur in storage even in the absence of common invasive forces such as birds, rodents, and insects. This spoilage is due to the immutable presence of fungal spores. Thus the only way to prevent spoilage is to avoid those conditions in storage that are conducive to fungal growth. This is not an easy feat however, because wheat often has a high moisture content and high temperature going into storage, due to weather conditions at the time of harvest.

Different options exist for the farmer after the wheat is harvested:

1) the wheat can be dried in a heated air dryer, then transferred to a bin and cooled by aeration,

2) the wheat can be dried and cooled directly in a bin with ambient air, or finally,

3) the farmer may choose to do nothing.

The resources of the farmer dictate which option is chosen. Many farmers may not have the equipment available for drying, or may not consider it cost effective due to the added energy costs involved. Even if the farmer chooses to dry, the wheat may still be at risk due to the slowness of the process. Some wheat may remain damp for a lengthy period before it can be dried, or rewetting of dried wheat may occur if the farmer passes air with a high relative humidity through it.

Many researchers have investigated the best means of optimizing the drying situation (Fraser 1979; Metzger and Muir 1983; Brook 1987; Sanderson et al. 1989; Sinicio 1994). A necessary component of this optimization is knowledge of the rate of deterioration of the wheat. By employing this information, the farmer can bring the wheat to safe storage conditions in time to prevent spoilage, without wasting any unnecessary energy costs. An equation that models the deterioration of wheat would be of particular value here.

A farmer who is unable to dry grain immediately however, is not left without options. The more information about the wheat that is available to farmers, the better able they are to decide what to do with it. An excellent resource for farmers are computer expert systems that can provide interactive information on drying and other elements of grain management (Mann 1995). Usually a wheat deterioration equation is incorporated into such systems, thus allowing the farmer to investigate different storage scenarios. Perhaps historical weather data indicate that the outside temperature should drop enough to cool the wheat in its current condition to a safe temperature. Or perhaps the farmer can sell it before the allowable safe storage time has elapsed. In either case the farmer could probably avoid drying.

It is preferable that a wheat deterioration equation model the condition of the wheat as its moisture content and temperature vary. This is a complex problem that is best undertaken by first breaking the object down into its component parts. Once these have been studied individually so that their impacts are understood they can be regrouped. In this study the main component considered was the effect that cooling would have on hard red spring wheat. I did not vary the moisture content to ensure that any effects would be due to cooling alone. A single moisture content of 17% (on a wet mass basis) was chosen for two reasons: 1) if weather conditions or harvesting strategies do not permit swathing, the wheat is often harvested at about this moisture content, and 2) many moulds are active at this level of moisture content. Future studies would need to repeat cooling tests at other moisture contents.

1.2 Objectives

The objectives of this study were:

1. To model the rate of reduction in the germination of hard red spring wheat stored at 17% moisture content over a period of 3 mo.

2. To develop a model of allowable storage time for hard red spring wheat at 17% moisture content under a) constant temperature, and b) decreasing temperature conditions during the 3 mo of storage following harvest.

3. To assess the usefulness of the deterioration parameters of visible mould and increasing free fatty acid content of the wheat as indicators of allowable storage time.

1.3 Assumptions inherent to the study

The assumptions made regarding the layout of this study were:

1. The prestorage life of the wheat used in this study would not significantly affect the outcome of the storage experiment.

2. The initial load of fungal spores in the wheat used, was typical of freshly harvested wheat and therefore representative of standard conditions.

3. The one cultivar of hard red spring wheat used in this experiment represents most cultivars of hard red spring wheat grown in Canada.

4. The small masses of wheat used in this experiment were valid representations of large masses in a bin.

5. There was enough consistency regarding the biological changes that occur in an ecological environment such as a grain bin that generalisations could be made.

2. LITERATURE REVIEW

2.1 Historical approaches to measuring grain deterioration

2.1.1 Deterioration parameters

For practical purposes, the only tenable parameter for a wheat deterioration equation is the point at which it drops in grade. This is the final designation of concern to the farmer wishing to sell this product. This designation, however, is somewhat obscure in that it does not adequately measure the quality of the grain. The Canadian Grain Commission determines the grade of grain through measurements of test weight, vitreousness, foreign material content, and a subjective determination of soundness (Canadian Grain Commission 1991). Such quality factors as odour, colour, and visible mould define soundness. This has resulted in researchers using numerous other quantifiable parameters of deterioration that are more indicative of the condition of the grain, and consistent in repeatability of results. Ultimately, as these parameters change they will indicate a condition that will affect the grading of the grain. Researchers have attempted to correlate these other parameters with degrading by the Canadian Grain Commission. Wallace et al. (1983) found that all seed having visible mould was musty, although all musty seed did not necessarily show visible mould. Mustiness and off-odours tended to be associated with storage fungi and with a decrease in germination, although germination was not strongly associated with degrading. Degrading, however, was often associated with high fat acidity values. So, while some obvious trends in evaluating deterioration exist, research to date has not defined them clearly. All these parameters, however, are linked to grain deterioration caused by the invasion of fungi.

One perspective is that because fungi cause deterioration, a measurement of the quantity of fungi present should give some indication of the magnitude of the deterioration

that has occurred. Quantifying fungi however, is an arduous task. Researchers have tried several methods, some of which include: counting fungal propagules through dilution plating (Bottomley et al. 1952; Golubchuk et al. 1956; Friday et al. 1986), counting the percentage of kernels infected in a sub-sample of the bulk (Wallace et al. 1962; Sinha 1983; Friday et al. 1986), applying a grading to the mould in the grain visible to the naked eye (Friday et al. 1986; Lacey et al. 1994), measuring the chitin content in the grain (Golubchuk et al. 1960; Wu and Stahmann 1975; Donald and Mirocha 1977; Nandi 1978), and measuring ergosterol levels in the grain (Seitz et al. 1977; Marfleet et al. 1991). Each of these approaches contributes some information that is distinct from the others, but each approach is limited by the narrowness of the information that it provides.

Partly due to the difficulties involved in direct enumeration of fungi, and partly due to the belief that quantification of the fungi alone is not definitive enough, the move in research has been toward measuring the byproducts of fungi to determine their impact on grain deterioration. A sample of the many methods includes measurement of: the decreasing germination of the seed (Kreyger 1972; Ellis and Roberts 1980; Wallace et al. 1983), the increasing respiration of the mould (Steele et al. 1969; White et al. 1982; Hamer et al. 1991; Wilcke et al. 1995), the rising electrical conductivity of the grain (Mills and Kim 1977, Sinha et al. 1981), the increasing formation of mycotoxins (Abramson et al. 1990), the decreasing falling number that occurs during flour milling (Bason et al. 1993), and the increasing free fatty acids (Sorger-Domenigg et al. 1955; Baker et al. 1957; Wallace et al. 1983; Juliano 1994).

Although changes in these factors all show deterioration in the grain, the quantity of change that corresponds to a drop in grade is not known. This suggests that none of these parameters is more useful than any of the others. Moreover, other conditions with respect to the history of the grain and the environment that one stores it in, combine to add degrees of complexity to such a measurement. Some important factors that researchers have

identified are: the prestorage conditions such as date of harvest (Thompson 1972, Ellis and Roberts 1980; Bason et al. 1994), the cultivar (Ellis and Roberts 1980; Stroshine et al 1986), the extent of mechanical damage (Steele et al. 1969), and the extent and type of initial fungal inoculum (Seitz et al. 1982a). To assess the contribution of each of these elements to the deterioration of the grain would add considerable complexity. Consequently, while some of these factors may be considered when modelling deterioration, many assumptions about the behaviour of the grain and the microorganisms living in it are still necessary.

2.1.2 Modelling deterioration of corn

Steele et al. (1969) developed the first useful mathematical equation to describe deterioration of a stored crop. Respiration from grain and the microorganisms living in it results in carbon dioxide (CO_2) formation. The researchers measured the CO_2 produced to depict the deterioration of shelled corn. They assumed that respiration occurred aerobically, accompanied by complete oxidation of carbohydrates:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + heat$$
 (2.1)

One should note however that such an assumption oversimplifies this phenomenon. Besides environmental conditions such as temperature and pressure, the consumption of oxygen (O_2) which occurs when fats are converted to carbohydrates or vice versa prior to oxidation must be considered (Milner and Geddes 1954). Later researchers have measured both O_2 consumption and CO_2 emissions, expressing the relationship as the respiratory quotient. In this way they have tried to adjust for any inadequacies in this assumption. In this early study by Steele et al. (1969) however, they measured CO_2 alone, and converted the quantity of CO_2 production to a loss in dry matter of the corn. Based on Eq. (2.1) they inferred that for every 1% loss in dry matter, 14.7 g CO_2 / kg of dry matter evolved. Saul and Steele (1966) had previously concluded that an acceptable maximum level of dry matter loss (DML) for field-shelled corn was 0.5%, as the grain could sustain this without being reduced in grade. They based their permissible storage time equation on the time it took to reach 0.5% DML under the conditions of 30% mechanical damage, 16°C and 25% moisture content (m.c.) on a wet mass basis (w.b.). The equation consisted of multiplication factors that modified this initial value to apply under different conditions:

$$T = \frac{T_R X M_T X M_M X M_D}{24}$$
(2.2)

where: T= storage time (d)

- T_R = time (230 h) resulting in 0.5% DML at 30% mechanical damage, 16°C and 25% m.c. (w.b.)
- M_T, M_M, and M_D are the reciprocals of ratios for the relative effect of temperature, moisture content, and mechanical damage respectively (as determined through empirical relationships).

The researchers made several observations about the effects of mechanical damage of the corn on deterioration. They noted that when mechanical damage increased, CO_2 production, and therefore DML, increased. The location of the damage on the corn also played a significant role. If the damage occurred to the embryo rather than the endosperm, it nearly doubled the rate of CO_2 production. Finally, even when they graded field-shelled corn as undamaged, it deteriorated at twice the rate of hand-shelled corn. This led the researchers to suggest that the common practice of visual grading of grain was inadequate.

Thompson (1972) mathematically simulated the effects of aeration and environmental conditions on high moisture shelled corn. Simulations consisted of varying airflows (1.3-26.4 $(L/s)/m^3$), harvest dates (Oct.1-Nov.15), initial moisture contents (20-28%), initial temperatures (-1, 10, 21°C), and official weather data for Nebraska. Thompson analysed the data from Steele et al. (1969) and modified their deterioration equation for use in his simulations. He related CO₂ production to time of storage under the standard conditions of

16°C, 25% m.c. , and 30% mechanical damage:

$$CD = 31.2(\exp(0.006T) - 1) + 0.36T$$
 (2.3)

where: $CD = g CO_2$ produced / kg dry matter.

His observations, based on the input weather data, were that higher airflows generally resulted in less grain deterioration, with the required minimum airflow rate equal to 13.2 (L/s)/m³. The date of harvest decreased deterioration by approximately 50%, for each 15-day delay after October 1. Grain deterioration was considered independent of initial grain temperature but highly dependent on initial moisture content. High temperatures increased drying initially, but could also increase deterioration rates. Lower initial temperatures would slow deterioration during storage, but total deterioration would still be the same. Alternately, high moisture contents doubled deterioration for each 2% increase in the range of 20-25% m.c.

Brooker and Duggal (1982) did similar tests on corn investigating the differences in allowable storage time as affected by heat buildup, natural convection, and aeration at an initial temperature of 15.6°C and initial moisture contents of 16 and 18%. As Thompson had observed, the airflow rate and the date of harvest combined to play a crucial role. At 16% m.c. an aeration rate of roughly 1.5 (L/s)/m³ maintained DML to a level less than 0.5% for approximately 180 days, whereas at 18% m.c. with natural convection they reached such losses in approximately 30 days. They noticed that when natural convection was used, 5°C changes could almost double spoilage rates. Whereas if aeration was used, the initial temperature had almost no effect because the grain was quickly cooled anyway. This was in agreement with Thompson's findings.

The distinction between CO_2 production by the grain or by the inhabitant mould is still unclear. Seitz et al. (1982a,b) monitored CO_2 production, ergosterol, aflatoxin, and percent

of kernels with fungi for freshly harvested corn at 22.9-25.6% m.c., to find a relationship between DML and fungal growth. They detected that the increase in the respiration rate did not correspond with the increase in fungi as indicated by ergosterol and aflatoxin contents, which suggested that much of the respiration was from the grain alone. Moreover, they discovered that aflatoxin production and fungal invasions could reach unacceptable levels before the corn reached 0.5% DML. The extent of the initial inoculum significantly affected the final fungal invasion though, which may account for differences between experiments. Finally, they confirmed that the location of damage on the grain affected the deterioration initially (the first 2-4 d), as earlier researchers had cited. Yet after 7 d all kernels that began with equal damage, whatever the position of the damage on the kernel, deteriorated similarly.

Contrary to other researchers Fernandez et al. (1982), in tests on corn at 26°C and moisture contents of 19 and 22%, found that CO_2 production did correlate well with the number of fungal propagules and percentage kernels infected. They introduced an equation based on the relationship between CO_2 and time, at the standard conditions of 26°C and 22% m.c.:

$$CD = -0.7646 + 0.4291 T + 0.0008966 T^2$$
 (2.4)

This equation agreed well with the equation of Steele et al. (1969) under the same conditions.

The major contribution of these studies was the first deterioration equation for a stored crop. This equation was based on accumulated CO_2 , which was converted to a theoretical DML during storage. Later researchers however, determined several flaws in this argument, such as unaccounted respiration by the grain itself, and unacceptable fungal invasion prior to 0.5% DML. The researchers agreed that DML increased with increasing mechanical damage, thereby eliminating visual grading as a sole indicator of quality. As well, they agreed that higher airflow rates through the stored crop, late in the harvesting season,

helped to eliminate the risks incurred by storing the grain at high temperatures early in the season.

2.1.3 Modelling deterioration of wheat

Fraser (1979), who was working on a simulation for solar grain drying, used Kreyger's (1972) data on the allowable storage time of wheat to model wheat deterioration. Kreyger himself however, is ambiguous about the source of these data. He omits to say how he obtained these data or even for what variety they are applicable. Despite this, Fraser lacking any other data to work with, derived a two-part model dependent on moisture content:

$$\log T = 6.234 - 0.2118m - 0.0527t \dots (12\% < m < 19\%)$$
(2.5a)

$$\log T = 4.129 - 0.0997 m - 0.0576 t \dots (19\% < m < 24\%)$$
(2.5b)

where: m = moisture content (%, w.b.) t = temperature (°C)

He defined the criteria for safe storage time as the time for grain to drop to 90-95% germination, or before mould growth became visible.

White et al. (1982) measured respiration in wheat. They determined an equation that yielded the rate of CO_2 production by microflora at temperatures ranging from 10 to 40°C, and moisture contents between 14 and 25% (w.b.). They measured CO_2 at predetermined intervals, after which they purged the test jars with compressed air. They related CO_2 production to independent factors such as temperature, moisture content, time, fat acidity value, germination, and microflora. From this they derived an equation to predict CO_2 production:

$$CD = (10^{-4.054 + 0.0406(t) - 0.0165(T) + 0.0001(T)^2 + 0.02389(m)})x(1000)$$
(2.6)

They determined values for DML from cumulative CO_2 values, which they calculated by summing CO_2 over time. The researchers listed equations relating cumulative levels of CO_2 to moisture content over time for each temperature. Using the allowable safe storage time predicted by Fraser's equation, they calculated the CO_2 that had accumulated at each temperature when this period had passed. According to this calculation, wheat being used for seed can have no more than 655 mg CO_2 / kg dry matter accumulation before it becomes unacceptable. According to their calculations this would correspond to a 0.04% DML. Alternatively, for the storage of wheat in general, an arbitrary value of 1470 mg CO_2 / kg dry matter accumulation, or a 0.1% DML, may be considered unacceptable.

Lacey et al. (1994) measured respiration of wheat as O_2 consumed. They observed that for temperatures of 15 to 35°C and moisture contents of 12.5 to 22.5% (w.b.), up to 0.13% DML occurred before wheat became visibly mouldy. While Wilcke et al. (1995) found that sound wheat stored at 18% m.c. and 20°C could experience 0.5% DML without any visible deterioration. However, at the higher moisture contents of 20 and 22% total damaged kernels and US grade worsened considerably by the time 0.5% DML had occurred.

Sanderson et al. (1989) used Fraser's equations to model deterioration during nearambient drying of wheat. Because the model was based on static conditions of temperature and moisture, it needed modification to apply it to a non-static situation, where drying was occurring. A deterioration index was introduced. For each time period that the grain bulk was under a different static condition they calculated the allowable storage time for that condition. The actual time spent at that condition was divided by the allowable time. They added this decimal fraction to the proportion of allowable storage time that had already elapsed. Once this value totalled to 1.0 they considered the predicted allowable storage time to have elapsed. Sanderson et al. (1989) determined however that the deterioration model was too conservative in its predictions and needed modification. Sinicio (1994) employed Fraser's (1979) equations to model wheat deterioration during aeration. He concluded that the uncertainty in the predictions on grain deterioration yielded by the equations, was greater than that in any of the other variables he employed in his model. He also suggested that further work was required in this area.

Although many researchers have gathered information on wheat deterioration, it is not conclusive with respect to allowable storage time. The source for the data used in the equations developed by Fraser is vague, and the application of the equations under changing conditions is dubious. Substantial information is available on CO₂ production and subsequent DML but it has not resulted in a loss equation. Difficulties about CO₂ build-up causing suppression of mould growth, and the applicability of the respiration equation under non-ideal conditions, add complexity and uncertainty to this measurement. All of the indices mentioned however, are inherently limited by the object that they are trying to measure. The deterioration of grain is a biological phenomenon that is unpredictable and not consistently repeatable. Under these circumstances allowances on accuracy are required. It is preferable to use indices that account for a variety of changes in the grain.

2.2 Stored grain deterioration parameters in this study

Sorger-Domenigg et al. (1955) suggest that if the storage moisture content is at a level which might indicate that present or future danger is possible, then a number of tests should be used in combination to get a clear picture of the deterioration occurring. Measurement of the number and kinds of mould present will indicate whether an invasion of the seed has already occurred. A decrease in viability signals whether incipient deterioration has developed, and the fat acidity value should give some measure of the actual damage which has already occurred.

A commonly used, albeit somewhat simple method of quantifying fungi, is through placing a sample of seeds on wet filter paper and counting the percentage of kernels

infected with fungi. This gives a gross idea of the degree of infection. Through identification of the fungi involved one can get an idea of the nature of the damage. Fungi require different microclimates for optimum growth. The group of fungi commonly referred to as the field fungi are usually present in freshly harvested wheat. They are so named because they invade the kernels before harvest, during plant growth or after cutting and swathing, but before threshing (Christensen and Kaufmann 1969). Once binned, if the storage conditions are poor, the group known as the storage fungi will begin to increase, inhibiting the field fungi. Grain that has been stored for some time yet has a high occurrence of Alternaria spp. accompanied by a low occurrence of storage moulds is indicative of good storage conditions. Although the species of fungi included in these two groups are vast in number, studies have identified those most commonly occurring in cereal grains. Alternaria spp. are the predominant field fungi, while Penicillium spp. and Aspergillus spp. are the predominant storage fungi (Wallace and Sinha 1962, Christensen and Kaufmann 1969, Mills and Wallace 1979). Alternaria spp. require at least 21-22% m.c. (w.b.) in starchy cereal grains to grow well (Christensen and Kaufmann 1969). They will however survive at low moisture contents (14-14.5 %) in storage without causing further damage. Depending on the temperature, and the specific species present, Aspergillus spp. will thrive in cereal grains at a moisture content of 14-18.5% (w.b.). Penicillium spp. require a slightly higher range of 16-20% m.c. (w.b.), although they can grow at much lower temperatures than most storage fungi (Semeniuk 1954, Sauer et al. 1992). If a cereal grain is held at a moisture content that falls in this range for more than a few days it is certain that some species of these microorganisms will develop. It is a further certainty that microorganism activity lowers the viability, storage qualities, nutritive value, edibility, and industrial usefulness of grain (Semeniuk 1954).

The germinability of grain is an important quality factor because it is vital to the continued production of the grain. Consequently, although grain may not be used for seed,

its germinability is a sensitive indicator of the deterioration of the grain (Pomeranz 1992).

Biochemical deterioration of grain can cause a loss in the nutritive value of the grain. This can occur through changes in carbohydrates, proteins, lipids, and vitamins (Pomeranz 1992). Changes in the fats can occur oxidatively or hydrolytically. In whole grain the latter is more common. When a hydrolytic change occurs, lipids break down into free fatty acids (FFA) and glycerol. Moulds can greatly accelerate this because of their high lipolytic activity. Thus it happens much more rapidly than the hydrolysis of protein or carbohydrates (Zeleny 1954). The measurement of FFA is expressed in terms of the mg of potassium hydroxide (KOH) required to neutralise the free fatty acids in 100 g of moisture-free seeds. This value is called the fat acidity value (FAV). Even though Baker et al. (1957) suggest that at 20 mg KOH/ 100 g grain all the wheat they examined was still of unquestionable soundness, researchers have not identified an absolute value to correspond to deterioration. Some researchers have avoided this complication by limiting themselves to expressing the increase in FFA in terms of a relative change (Sinha 1983). This way, the magnitude of the change in the FFA can readily be associated with a relative change in deterioration.

3. MATERIALS AND METHODS

3.1 Experimental setup

3.1.1 Experimental design

I designed this experiment to reproduce the deterioration of wheat as it would occur in storage during the first 3 mo after harvest. Due to time and resource limitations I could not test all Manitoba wheat cultivars, nor all storage conditions concerning temperature and moisture content of the wheat, as I would require for a complete analysis of this topic. Instead I planned a more thorough study of one element of this topic. This could lead to a broader yet more directed study in the future. I restricted myself to testing hard red spring wheat seed (Triticum aestivum L., cv. 'Katepwa', harvested 1994) at a moisture content (m.c.) of 17%. I selected this moisture content because the fungi detected in the grain, during a preliminary plating, would thrive at this condition. Data from Manitoba Agriculture for the span of 1986-95 show that harvesting of spring wheat generally begins in mid-August and often continues through to the beginning of October. Local weather data (Environment Canada) for the years 1985-94 show that the mean 24 h temperature over these weeks is 14°C, with the maximum sometimes reaching 35°C. Prasad et al. (1978) found that the temperature of wheat in swath is ca. 8°C higher than the surrounding ambient temperature when it is harvested. Therefore, I chose storage temperatures from 15 to 35°C, in 5°C intervals, to cover a realistic range. The temperature schedules selected consisted of decreasing temperatures only, because ambient conditions surrounding grain bins predominantly decrease in the autumn months. The study covered a 12 wk span, so that from the earliest harvest date this would extend into mid-November, by which time the outdoor temperatures decrease considerably and farmers generally do not operate drying fans.

Space in the environmental chamber, and more significantly the number of channels available for monitoring by the data acquisition system, limited the number of tests that I could conduct at one time. The system acquired for this study had 48 channels for reading inputs and 12 channels writing outputs. In addition, I applied the restriction of three replications for each test. I conducted two sets of tests examining a total of 24 temperature schedules (Table 3.1) from 15 to 35°C, with Test 1-1 repeated in the second set due to excessive drying of the grain during the first set. The first set of tests began on July 18, 1995 and were completed on October 10, 1995. The tests ended prematurely after 84 d because the data acquisition system controlling the environmental conditions ceased to operate. Only seven days were remaining in the first trial though, and all the samples had already deteriorated beyond the predetermined limit. The second set of tests began on November 21, 1995 and were completed on February 20, 1996, after 91 d.

Table 3.1.	Temperature	schedule
------------	-------------	----------

Test set no.1 (84 d)	Test set no.2 (91 d)
1 = 35°C - d. 1-84 (repeated in 2nd set) 2 - 30°C - d. 1-84 3 - 25°C - d. 1-84 3 - 25°C - d. 1-84 4 - 20°C - d. 1-84 5 - 15°C - d. 1-84 6 - 35°C - d. 1-84 6 - 35°C - d. 1-4 / 25°C - d. 5-84 7 - 35°C - d. 1-4 / 20°C - d. 5-84 8 - 30°C - d. 1-7 / 25°C - d. 8-14 / 20°C - d. 8-14 / 20°C - d. 15-84 9 - 30°C - d. 1-7 / 20°C - d. 8-21 / 15°C - d. 22-84 10 - 30°C - d. 1-7 / 20°C - d. 8-84 11 - 25°C - d. 1-14 / 20°C - d. 15-84 12 - 25°C - d. 1-7 / 20°C - d. 8-35 /	Test set no.2 (91 d) 1 - 35° C - d. 1-91 2 - 35° C - d. 1-21 / 25° C - d. 22-91 3 - 35° C - d. 1-21 / 20° C - d. 22-91 4 - 35° C - d. 1-21 / 25° C - d. 22-56 / 15^{\circ}C - d. 57-91 5 - 30° C - d. 1-21 / 25° C - d. 22-56 / 20^{\circ}C - d. 57-91 6 - 30° C - d. 1-28 / 15° C - d. 29-91 7 - 30° C - d. 1-28 / 20° C - d. 29-91 8 - 30° C - d. 1-28 / 25° C - d. 29-91 9 - 30° C - d. 1-28 / 25° C - d. 29-91 9 - 30° C - d. 1-28 / 20° C - d. 29-56 / 15^{\circ}C - d. 57-91 10 - 25° C - d. 1-28 / 20° C - d. 29-56 / 15^{\circ}C - d. 57-91
15°C - d. 36-84	11 - 25°C - d. 1-42 / 20°C - d. 43-91
	12 - 35°C - d. 1-21 / 15°C - d. 22-91



Figure 3.1. Experimental chamber with apparatus.

3.1.2 Experimental apparatus

I held the experiment in a CRELAB (Climatic research equipment, WHL3 - 610M, Winnipeg, MB.) environmental chamber. Within the chamber were eight Styrofoam boxes (Fig. 3.1) which could each house a maximum of two sets (three or six replicate containers). A Datascan 7010 data acquisition system (Firmware v2.0 Measurement Systems Ltd., Newbury, Berkshire, UK), operated by a BASIC software program on a Tandy computer, controlled the complete system. The data acquisition system read one thermocouple from each sample container (totalling 36), one thermocouple from each insulated box (totalling 8), and two thermocouples from the chamber. The data acquisition system controlled eight 1500 W heaters, one housed in each of the insulated boxes, and the heating/cooling switch of the environmental chamber. The chamber was always set to a temperature 5±3°C below the lowest temperature of any of the boxes. The heater in each box acted as the heat source required to raise the temperature of the box to the desired temperature above its surroundings inside the chamber. The program permitted only two heaters to operate at any time. It computed the temperature of each box by calculating the mean of the temperatures indicated by the thermocouple in the box and the thermocouples in the test containers in the box. The mean temperature of each box was checked every 10 s. The heaters for the two boxes furthest from their desired set point temperature were powered on. In this way the system maintained the temperature of each sample container within ±2°C of its desired set point temperature. I increased the relative humidity in the boxes by placing a dish of distilled water in front of each heater.

I placed the grain in cylindrical galvanised steel containers (Fig. 3.2). The bottom of each container consisted of a wooden plug with 31 ventilation holes and the top was a plastic lid. This arrangement of each cylinder allowed movement of air through the container similar to that in a bin, creating a realistic condition for fungal growth. As fungi grow they respire,



Figure 3.2. Individual test containers holding grain bulks.

emitting CO_2 . In a completely enclosed environment CO_2 would accumulate, replacing O_2 , and causing inhibition of fungal growth. Each test container held 2000 g of wheat, which was divided into four mesh bags layered atop one another. The inner layers acted as the actual experimental sample. The top and bottom layers served to deter moisture diffusion away from the experimental sample, being replaced if their moisture content began to decrease. I moved the test containers to a box of the appropriate temperature as necessary throughout the experiment.

3.1.3 Sampling frequency

I removed representative samples of 40 g from each of the 36 test containers once each week. At the time of sampling I thoroughly mixed the grain bulks, and if they were drying out because of moisture diffusion and free convection currents, I also misted them with distilled water. Although samples were removed once every 7 d, laboratory analysis was done only on samples collected every 14 d. The exceptions to this were few:

a) In the first set I took samples on d. 4. This decision was based on Fraser's prediction that deterioration at 35°C would occur by d. 6. Because of this significant changes were expected by d. 4.

b) Visible mould detection was done through simple observation once every 7 d when the samples were removed.

c) Sometimes the rate of change in germination or fat acidity value (FAV) occurred too rapidly over a 14 d period to yield explicit results. In these cases I analysed the mid sample (a 7 d interval) to ascertain more precisely the time of change.

3.2 Laboratory analysis of data

3.2.1 Preliminary condition of sample

I received the wheat directly from a farm in May of 1995, where the farmer had stored it since the previous year's harvest. A preliminary sampling of the grain showed that upon arrival it had a mean m.c. of 12.1% and a mean germination rate of 99%. An inspection of the fungi present showed that infection with *Alternaria* spp. was high (ca. 91%), but no infection with any storage fungi was apparent. On the morning that each of the two trials began I conditioned the wheat to 17±1% m.c. by adding distilled water. I determined moisture content on a wet basis, using the oven method outlined in the ASAE Standards (ASAE 1993). Ten grams of unground wheat were dried at 130±1°C for 19 h.

3.2.2 Germination

I tested the germinative capacity of the samples by placing seeds on water saturated filter paper in two petri dishes with 25 kernels apiece from each sample (Wallace and Sinha 1962). I lined the petri dishes with Whatman no.3 filter paper, and wetted them with 5.5 mL of distilled water. I placed the dishes on racks inside plastic bags and stored them in an incubation chamber at 25°C. On the fourth day of incubation I removed the plastic bags, and on the seventh day I counted the number of germinated seeds.

3.2.3 Microflora identification

I checked for microflora growth in the samples by plating four petri dishes, lined with Whatman no.3 filter paper, with 25 kernels each. On two of these I employed the filter paper (FP) method (Wallace and Sinha 1962), by wetting the dishes with 5.5 mL of distilled water. On the other two I employed the salted filter paper (SFP) method (Mills et al. 1978), by wetting the dishes with 5.25 mL of a solution of 7.5% NaCl in distilled water. I placed the dishes on racks inside plastic bags and stored them in an incubation chamber at 25°C. The lighting schedule of the incubation chamber followed a 24 h pattern: 12 h of white fluorescent lights then 12 h of white fluorescent lights and ultraviolet lights together. On the fourth day of storage I removed the plastic bags, and on the seventh day I identified the microflora that had grown on the seed.

3.2.4 Visible mould detection

Using the naked eye I observed the grain at each sampling interval to detect any visible mould. This is a highly subjective method that only supplements the more analytical techniques described above. It is a useful indicator of when deterioration has begun. I used the following index:

- 0 no visible mould growth
- 1 visible mould on a few kernels at periphery of bags (hereafter referred to as the first appearance)
- 2 visible mould throughout the bulk of the sample
- 3 sample beginning to turn grey or dull in colour
- 4 sample grey or dull in colour
- 5 sample extensively damaged.

3.2.3 Free fatty acid detection

I determined the free fatty acid (FFA) value based on the procedure outlined by the American Association of Cereal Chemists (AACC 1962), with modifications according to Demianyk (1995). Samples were dried at 130°C for 19 h, then ground in a Tecator cyclone sample mill. I placed 5 g of the ground wheat in Whatman no.5 filter paper, which was folded and placed inside aluminum cylinders. I fastened the cylinders to a Goldfisch fat/oil extractor (LabConco Corporation; Kansas City, MO; 115 V, 5.2 A, phase 1, Cycle 50/60)

inside beakers containing 30 mL of petroleum ether. The petroleum ether was boiled and condensed through the samples for 6 h. Following this, the petroleum ether was vaporised and I added 25 mL of TAP solution (50% toluene / 50% ethanol). Using a KOH solution consisting of 1.1979 mg KOH / mL solution, I titrated this until it was neutralized and just turned pink. The FAV was expressed as mg of KOH required to neutralize the FFA in 100 g of dry grain.

inite in the second

4. RESULTS

4.1 Rate of reduction in the germination over time

A plot of the germination (data in Appendix A) over 84 days at each of the five constant temperature conditions showed that the data followed an asymmetric sigmoid pattern. I have described this pattern using the following logistic function with five parameters (Sigma Plot 1988):

$$f(x) = \frac{a - d}{\left[1 + \left(\frac{x}{c}\right)^{b}\right]^{e}} + d$$
 (4.1a)

where: a = asymptotic maximum b = slope parameter c = x value at the inflection point d = asymptotic minimum e = symmetry parameter

Ideally the asymptotic maximum should be 100, representing the theoretical maximum germination. In a preliminary sampling of the wheat however, I obtained a germination rate of 99%. At the start of the experiment, after moisture conditioning, the mean germination rate was 96%. I thus selected a value between these two, of 98%, to represent the asymptotic maximum (parameter a) for this model. Functionally the minimum value for germination must be zero. Therefore, the asymptotic minimum (parameter d) was forced to zero. Employing the software package Statistica (STATISTICA) I applied the quasi-Newtonian estimation method to this equation to determine the remaining parameters b, c, and e for each of the five constant temperature conditions. In the quasi-Newtonian estimation method the partial derivatives of the loss function are asymptotically estimated and then used to determine the movement of parameters from iteration to iteration.

The equation that I developed to model the rate of reduction in the germination of the wheat, at the five constant temperatures, is:

$$G = \frac{98}{\left[1 + \left(\frac{T}{c}\right)^{b}\right]^{e}}$$
(4.1*b*)

where: G = germination rate (%) T = storage time (d); valid for 1-84 d.

A different set of equation coefficients was determined for each temperature (Table 4.1).

Table 4.1.	Storage	temperature	coefficients	for E	q. (4.1)	
------------	---------	-------------	--------------	-------	------	------	--

	Storage temperature				
Coefficient	35°C	30°C	25°C	20°C	15°C
b (Slope parameter)	3.3	4.0	6.0	5.0	8.0
c (Inflection point)	19	20	23	60	160
e (Symmetry parameter)	1.3	0.51	0.15	0.50	75
R ²	0.997	0.996	0.925	0.975	0.932

In Fig. 4.1, I plotted the equations with the corresponding means of the experimental values. Each experimental data point shown represents from 3-18 observations, depending on the number of tests that were combined to calculate the values at each temperature (values with standard deviations in Table A.3, Appendix A).



Figure 4.1. Changing germination of wheat at 17% moisture content and five constant temperature conditions[†].

[†]points represent experimental values; curves represent the values as plotted using the fitted equations

4.2 Allowable storage time equation

4.2.1 Constant conditions

The deterioration parameter that I chose to define the allowable storage time before an unacceptable level of deterioration occurred was germination. Other researchers have chosen markers such as a 5-10% drop (Fraser 1979) or a drop to 85% (Wallace et al. 1983), but to obtain conservative results, I chose a drop to 90% germination for this study.

Five allowable storage times at constant conditions were determined, one at each temperature studied. I determined these times by pooling the three replicate values from the constant temperature tests with related values from any other tests kept at the same initial
temperature for a prolonged period. In this way I could consider the maximum amount of data in each case. The tests considered in determining the allowable storage time at 35°C are shown in Table 4.2.

Set - Test [†]	Days
2-1	1 - 84
2-2	1 - 21
2-3	1 - 21
2-4	1 - 21
2-12	1 - 21

Та	ble 4.2.	Tests us	ed to	determine	storage	time at 35°C.	
----	----------	----------	-------	-----------	---------	---------------	--

[†] Test conditions given in Table 3.1, Section 3.1.1.

I used the final storage values obtained for the five temperatures to develop an equation for allowable storage time. I decided to base this model on Fraser's equation, Eq. (2.5a), so I tested the same generic function with the moisture content factored out:

$$f(x) = 10^{a - bx}$$
 (4.2a)

using the software package Statistica (STATISTICA). The resulting prediction equation for the allowable storage time of wheat at 17% moisture content (m.c.) was:

 $T = 10^{2.5 - 0.045 t} \tag{4.2b}$

where: t = temperature (°C); valid between 15 and 35°C.

The coefficient of determination for this equation was 0.997. The allowable storage times predicted by this equation, Fraser's equation, and the observed data are shown in Fig. 4.2. No appreciable differences exist between the new equation and Fraser's equation.



Figure 4.2. Allowable storage times of wheat at 17% m.c.

4.2.2 Comparison of first and second sets of experiments

The overall deterioration of the wheat in the second set of tests appears to be more marked than in the first, although I have not determined any definite reason for it. I have illustrated this in Fig. 4.3, in a comparison of tests from the two sets that have the same temperature up to day 42 but have markedly different results. In the example from the first set, germination did not decrease to 90% until about day 37, whereas in the example from the second set it had decreased to 90% in less than half that time, that is, by day 15 (Fig. 4.3a and b). Microflora readings on the conditioned wheat at the beginning of the second set of tests showed that percent infection by storage moulds was nil, but percent infection by field moulds had decreased since the first set of tests was begun (Fig. 4.3c and d). In the examples shown here *A. glaucus* gr. increased at similar rates in the two tests. The



Figure 4.3. Comparison of deterioration samples in set 1 and 2 with a similar temperature schedule.

Germination comparison: (a) Test 1-3: 25°C for d.1-84; Microflora comparison: (c) Test 1-3: 25°C for d.1-84; (d) Test 2-11: 25°C for d.1-42 followed by 20°C for d.43-84; (d) Test 2-11: 25°C for d.1-42 followed by 20°C for d.43-84.

Penicillium spp. however showed up earlier, by day 21 in the second set, compared with the end of the test (d. 84) in the first set. Two possible explanations for the discrepancies between the sets are:

1) The grain may have undergone slow deterioration while it was in cool storage for 4 mo between tests. During this time I stored it at 10°C and an initial m.c. of 12%, that rose to almost 14% due to the humidity of the surrounding air. However, despite the changes that occurred in the second set of tests, inspection of the microflora on the wheat in cool storage at the end of the second set indicated that no storage moulds had appeared yet. Moreover, infection of the wheat by field fungi after 9 mo in cool storage was still high. Also, the wheat had been in on-farm storage for approximately 9 mo prior to this experiment, the effects of which are unknown.

	Moisture Content (%, w.b.)							
Test	Set 1	s.d.	Set 2	s.d.				
1	-	-	16.5	0.7				
2	16.6	1.0	16.8	0.6				
3	16.6	0.4	16.9	0.6				
4	17.1	0.5	16.9	0.5				
5	17.1	0.4	17.3	0.8				
6	16.4	0.6	17.1	0.9				
7	16.7	0.4	16.9	0.7				
8	17.1	0.9	16.6	0.6				
9	16.7	0.4	16.9	0.5				
10	17.0	0.8	17.4	0.7				
11	16.9	0.5	17.6	1.1				
12	16.8	0.6	17.4	1.5				

Table 4.3. Mean moisture content of each test.

2) I periodically misted and mixed the grain during the second set of tests to keep the m.c. from decreasing too much. Although I believe that the moisture was transferred throughout the bulk, it may be possible that the initially high moisture content on some kernels had a detrimental effect. Although there were variations in the moisture content of the grain throughout the duration of the tests (data in Appendix F), the overall mean for each test (Table 4.3) throughout the experiment remained close to 17%.

The sets were distinct from each other due to the inexplicable biotic differences between them, and the differences in the lengths of time that they sustained high temperatures at the beginning of the tests. For these reasons the two sets were considered separately when discussing the trends for decreasing temperature conditions. Because Eq. (4.1) and Eq. (4.2) are based on data from both sets of tests however, when the two sets are independently compared to the results from these equations they each appear skewed.

4.2.3 Decreasing temperature conditions

In the first set, for the tests that were decreased in temperature, I selected the length of the storage period at the initial temperature based on Fraser's prediction. That is, I selected the length of the storage period at the initial temperature so that apparent deterioration should have followed almost directly after the temperature change. Under these variable temperature conditions however, deterioration to 90% germination occurred much later than predicted (Fig. 4.4).

An example of how this deterioration index is employed using Test 1-8 follows. Test 1-8 had a temperature schedule of 30°C for days 1-7, followed by a decrease to 25°C for days 8-14, followed by a decrease to 20°C for the remainder of the test. Using Fraser's equation, Eq. (2.5a), to determine the allowable storage time at any given temperature:

Predicted allowable storage time at 30° C: 11 d Actual experimental time at 30° C: 7 d Fraction of storage life elapsed after 7 d: 7/11 = 0.64

Predicted allowable storage time at 25°C: 21 d Actual experimental time at 25°C: 7 d Fraction of storage life elapsed after additional 7 d: 7/21 = 0.33

Total fraction of storage life elapsed after 14 d: 0.64 + 0.33 = 0.97Fraction of storage life remaining after 14 d: 1.0 - 0.97 = 0.03

Predicted allowable storage time at 20°C: 38 d Number of days for remaining storage life at 20°C: $0.03 \times 38 \text{ d} = 1 \text{ d}$ Total allowable storage time at this temperature schedule = 7 + 7 + 1 = 15 d

Sanderson et al. (1989) suggested that Fraser's predictions were inaccurate when applied to bin conditions where ventilation or near-ambient drying occurred because they did not account for the effects of changing temperatures or moisture contents. They suggested that the concept of a deterioration index (DI) could be used but that it should be raised to a value higher than 1.0 in these circumstances.

Using Eq. (4.2b) with a DI of 1.4, I predicted new allowable storage times (Fig. 4.4). This method predicted allowable storage times that more closely matched the experimental data. For the most part however, the predictions still remained on the conservative side, reducing the risk of spoilage.

In planning the second set of tests based on the results of the first set of tests, I substantially lengthened the storage period at which the wheat remained at a high temperature. The intention was to hasten deterioration so that it would occur prior to when it occurred in the first set. This was effective in causing a change, but resulted in excessive deterioration within the high temperature storage period, before any change in the temperature. Consequently, applying the methodology mentioned above for predicting allowable storage times during changing temperatures was impossible. I examined a different method however, for predicting germination. Using Eq. (4.1b) (Section 4.1), the germination after a fixed period of time at a given temperature can be determined. I



Figure 4.4. Comparison of predicted and measured allowable storage times for tests with changing temperatures.

* Temperature schedules for these tests are in Table 3.1.

** Prediction made with Eq. (2.5a).

*** Prediction made with Eq. (4.2b).

hypothesized that if this equation was applied in sequence for each change in temperature the germination could be determined at any time for any temperature schedule. Many tests that underwent a decrease in temperature however, decreased in germination beyond the level of the constant temperature tests within the 84 d time frame. As the prediction equations are only valid for up to 84 d (through experimental verification) I could only test this hypothesis on the four tests (2-5, 2-8, 2-10, and 2-11) that fit within these parameters (Fig. 4.5). For example, Test 2-5 had a temperature schedule of 30°C for 21 d, followed by 25°C for another 35 d, followed by 20°C for the remainder of the test. A sample calculation for the predicted results in Test 2-5, 1 wk after the first temperature change follows:

Theoretical germination at the start: 98%





(a) Test 2-5; (b) Test 2-8; (c) Test 2-10; and (d) Test 2-11 (Section 4.1, Table 4.1) ------- experimental data

----e---- predicted prior to temperature change (Eq. 4.1b)

----o---- predicted following temperature change (Eq. 4.1b).

Following the curves in Fig. 4.1: predicted germination after 21 d at 30°C: 65% Moving horizontally to the curve for 25°C: equivalent time required to reach 65% germination at 25°C: 42 d

Predicted germination after an additional week at 25° C (42 + 7 = 49 d): 56% Actual experimental germination one week after change in temperature : 40%.

Although Test 2-8 almost seems to fit the theory, it is obvious from the poor results of the other three that some factor of deterioration is unaccounted for here. In Tests 2-5 and 2-8 there is good correspondence between the observed and predicted data until the temperature change, after which the predicted data underestimates the germination loss that occurs. It seems that the effect of the higher temperature continues for some time after the grain is cooled. Whereas in Tests 2-10 and 2-11, the experimental data undergoes more deterioration than the predicted after the first 2 wk.

4.3 Correlation of other deterioration indices to loss in germination

4.3.1 Correlation of microflora activity to decrease in germination

Visible mould appeared after the experimental germination had already dropped below 90% in 20 out of 23 tests (Table 4.5, and Appendix B). The mean number of days at which its appearance followed the decrease in germination was 4.7 d. This difference will be somewhat greater than the actual occurrence because I made observations on visible mould only once every 7 d. For the constant conditions, excepting the lowest temperature, visible mould appeared within one standard deviation of the time that it took for the germination to decrease to 90% (Fig. 4.6).

I identified microflora using the two methods of SFP (data in Appendix C) and FP (data in Appendix D). My discussion of microflora however, is limited to the results from the SFP method because it yielded greater fungal growth. Overall, infection with *Alternaria* spp. was quite high at the start, while infection with storage fungi was not evident. Due to

changes in the storage temperature and apparent competition between the species, as the *Alternaria* spp. population began to decrease the *A. glaucus* gr. population began to increase, followed by an increase in the *Penicillium* spp. population (Fig. 4.7). By the time visible mould had appeared, infection with *A. glaucus* gr. was roughly 60% or higher, *Alternaria* spp. had decreased considerably, and *Penicillium* spp. were just beginning to show, or had not yet even appeared. Hence, since *A. glaucus* gr. was the predominant storage fungi in most tests, this is likely the mould that was visible to the naked eye. The greater than 60% infection of the grain with *A. glaucus* gr. prior to the appearance of visible mould, explains why the germination had decreased to below 90% before this time. This corresponds to a proposal by Sauer et al. (1992), in which they state that by the time grain

Set - Test	Germination drop to 90% (d)	Visible mould appearance (d)	Set - Test	Germination drop to 90% (d)	Visible mould appearance (d)
1-1	-	-	2-1	8	14
1-2	36	42	2-2	11	14
1-3	37	42	2-3	3	14
1-4	44	49	2-4	2	14
1-5	68	77	2-5	11	21
1-6	41	49	2-6	16	21
1-7	52	49	2-7	10	14
1-8	40	42	2-8	15	14
1-9	58	63	2-9	14	21
1-10	47	42	2-10	15	21
1-11	42	42	2-11	15	21
1-12	49	49	2-12	8	14

 Table 4.4.
 Time of the initial onset of deterioration as defined by a decrease in germination and the first appearance of visible mould.



Figure 4.6. Allowable storage time as defined by germination drop[†] compared with the first sign of visible mould.

[†]bars indicate one standard deviation

3

has been more than 40% infected with A. glaucus gr. it is of questionable soundness.

4.3.2 Correlation of an increase in the fat acidity value with a decrease in germination

At the lower temperatures there was a minimal increase in the fat acidity value (FAV), whereas at the higher temperatures the increase was quite dramatic and sudden (Fig. 4.8, and Appendix E). The decrease in germination to 90% seems to occur just before the obvious increase in FAV (Fig. 4.8). This is a logical progression. As moulds move into the grain, they attack the embryo thus terminating any potential for germination. Moulds do not thrive well on fat (Wallace 1973) but their added lipolytic activity eventually causes the





Figure 4.7. Microflora examples with visible mould appearance and germination decrease to 90%.

(a) Test 1-9: 30°C for d. 1-7 / 20°C for d. 8-21 / 15°C for d. 22 - 84 (b) Test 2-10: 25°C for d. 1-28 / 20°C for d. 29-56 / 15°C for d. 57-84.



Figure 4.8. Change in the fat acidity value at constant conditions.

breakdown of the fats in the grain into free fatty acids (FFA).

Although Baker et al. (1957) suggested that grain, having reached a FAV of 20 mg KOH/100g grain is still sound, it is uncertain how they defined sound. In all of my tests the grain had deteriorated well below a germination of 90% before FAV increased to 20 mg KOH/100g. Due to the different FAV that can result from the activity of different moulds, I decided that it would not be practical to try to obtain an absolute value to which to equate deterioration. Instead I took an alternate approach, expressing the increase in FAV as a change relative to its start value. The marker I used was the percent increase in FAV that corresponded most closely to a decrease in germination to 90%. This marker was a 25% increase from its start value (Fig. 4.9). The data used are from the second set of tests and from the tests held at constant conditions in the first set.

The coefficient of determination for the linear regression is 0.914. This suggests that a 25% value could be used as a general guide for wheat at 17% m.c.



Figure 4.9. Correlation of the allowable storage time as defined by a 25% increase in FAV to the allowable storage time as defined by a decrease to 90% germination.

5. DISCUSSION

5.1 Rate of reduction in the germination of wheat

In this study I determined that the rate of reduction in the germination of wheat at 17% moisture content (m.c.) could be modelled using an asymmetric sigmoid equation. Confirmation of this pattern for wheat deterioration parameters is provided by Lacey et al. (1994) who suggest that respiration-time relationships are also sigmoidal (inverse of germination sigmoid). They suggest that transformation of the grain starts with an initial lag phase. For microorganisms this is the stage when the spores existing in the grain acclimate, and those that can thrive in this environment begin to grow. An exponential phase follows this, when the microorganisms multiply. Finally a plateau or declining phase ensues when nutrient reserves are exhausted causing an impedance to further growth of the microorganisms. The initial lag phase becomes less apparent at higher temperatures due to the more rapid onset of deterioration under these conditions, therefore causing asymmetry. The sigmoid pattern for germination is consistent for other crops as well.

Some of the crops that this pattern has been identified for (Fig. 5.1) include: barley (Roberts and Abdalla 1968), soybeans (Dorworth and Christensen 1968), onion seed (Siegenthaler and Douet-Orhant 1994), and broad beans (Roberts and Abdalla 1968). For each crop the germination remains high until a period of sudden decay commences. Once the germination potential is almost exhausted, the rate of decay tapers off so that germination slowly approaches zero. Eq. (4.1) is effective in modelling the data for these crops (Table 5.1). Assessing which factors are contributing most to the differences in the resulting coefficients is difficult. Barley and soybeans behave similarly at 25°C with comparable water activity (0.87 and 0.81 respectively). The exception is that soybeans, with a high coefficient for slope, deteriorate more rapidly in the mid-phase. This can only be due



Figure 5.1 Deterioration curves for various crops in storage signifying that germination change is commonly sigmoidal.

- (a) Barley stored at 25°C and 18% m.c. (Roberts and Abdalla 1968)
 (b) Soybeans stored at 25°C and 16.5% m.c. (Dorworth and Christensen 1968)
- (c) Onion seed stored at 30°C and 9% m.c. (Siegenthaler and Douet-Orhant 1994)
- (d) Broad beans stored at 35°C and 18.5% m.c. (Roberts and Abdalla 1968).

	(sto	Crop (storage temperature; moisture content)						
Coefficient	barley (25°C; 18%)	soybeans (25°C; 16.5%)	onion seed (30°C; 9%)	broad beans (35°C; 18.5%)				
b (slope parameter)	8.2	13	4.2	5.4				
c (inflection point)	65	65	280	19				
e (symmetry parameter)	0.53	0.64	0.75	1.3				
R ²	0.994	0.999	0.976	0.998				

Table 5.1. Varying crop coefficients for Eq. (4.1) (Section 4.1).

to the differences in the physical make-up of the two crops. Neither crop however, compares well with wheat (Section 4.1, Fig. 4.1) which did not deteriorate nearly as much in the same time span. Wheat and onion seed at 30°C have a similar pattern except that the inflection point for the onion seed is greater by a factor of 10, corresponding to the longer time over which it deteriorated. Finally, the broad beans at 35°C compare well with wheat at the same temperature except for a higher coefficient for slope. In all these cases however, ascertaining whether the differences are due to storage conditions or crop type is impossible with the available information.

5.2 Allowable storage time

5.2.1 Constant conditions

The allowable storage times for wheat under constant moisture and temperature conditions predicted by Eq. (4.2b) (Table 5.2) supports Harrington's (second) rule of thumb (Harrington 1963), that each increase in temperature by 5°C roughly reduces the storage life by one half. Brooker and Duggal (1982) found similar results for corn (Section 2.1.2).

Wallace et al. (1983) used a deterioration parameter of 85% germination to define

Temperature	Storage Time					
°C	Eq. (4.2b) prediction	Harrington's rule of thumb				
20	40	40				
25	24	20				
30	14	10				
35	8	5				

Table 5.2.	Storage time of wheat roughly halved for every increase in
	temperature by 5°C.



Figure 5.2. Allowable storage time of wheat from Wallace et al. (1983) at two sets of moisture contents, with prediction Eq. (4.2b) (Section 4.2).

their allowable storage time. Despite this value being slightly less conservative than the 90% germination limit used here, a plot of their suggested allowable storage times (Fig. 5.2) confirms the validity of the new storage prediction equation, Eq. (4.2b), derived herein. They based their values on experiments conducted on six different lots of wheat stored in 3.6 L jars in the laboratory. They assessed quality with respect to microflora, germination, FAV, and grading by the Canadian Grain Commission. Combining these data they yielded an interval of time during which the grain's allowable safe storage period would expire. It seems that for the moisture range studied the allowable storage time is affected more sharply by differences in the moisture content at lower temperatures than at the higher temperatures.

Trisvyatskii (1969) provides data for 20°C and below only (Fig. 5.3), predicting shorter storage times than those predicted with Eq. (4.2b). Discerning the conditions of his experiment and the type of wheat to which the data are applicable however, is difficult.

Mills (1992) based his guidelines for safe storage on findings by other researchers. He proposed a very broad band of allowable storage times (Fig. 5.3). He did not show how he came to this result, but presumably he was intentionally vague to account for the many definitions of safe storage. Perhaps this is the most realistic approach considering the variability inherent in biological models. The objective of my study however, was to produce a narrower definition of allowable storage time so that it could be applied to more precise applications such as in computer models. The new prediction equation, Eq. (4.2b), appears to be compatible with the findings of the researchers discussed above. Thus, despite the differences in the deterioration rate between the two sets of tests, their combined mean yields realistic data.

5.2.2 Decreasing temperature conditions

Although I have suggested using a deterioration index of 1.4 in conjunction with Eq.



Figure 5.3. Allowable storage time of wheat from Trisvyatskii (1969) and Mills (1992) with prediction Eq. (4.2b) (Section 4.2).

(4.2) to calculate the allowable storage times for grain at decreasing temperatures, this is not a true depiction of what is occurring. That is, despite the actual sigmoidal pattern of the change in germination, the inherent assumption in these calculations is that it is linear from 98% to 90% germination (Fig. 5.4). This assumption is a necessary simplification so that one can use a single equation to cover a range of temperatures. It is obvious in examining the slope of the linear curve at each temperature compared with the actual germination curve, that as the temperatures are lowered, the differences between the linear and sigmoidal curves increase. This means that the predictions based on the linear curves are not as conservative as those that would be based on the sigmoidal curves. An example using Test 1-7, kept at 35°C for 4 d and at 20°C for the remainder of the test, follows:

Using the linear prediction curve: Maximum storage life at 35°C: 8 d The storage life elapsed after 4 d: 4/8=0.5; germination: 94%.

Reduce temperature to 20° C. Maximum storage life at 20° C: 43 d Storage life remaining: 0.5 x 43 d = 21 d Total storage life estimated to be: 4 + 21 d = **25 d**.

In comparison on the sigmoidal prediction curve: Germination after 4 days at 35°C: 97%.

Reduce temperature to 20°C. At 20°C 97% germination occurs after: 28 d Storage life remaining: 43 - 28 = 15 d

Total storage life estimated to be: 4 + 15 d = 19 d.



Figure 5.4. Germination from the start of storage to the 90% germination cut off for allowable storage time.

Because the "true" germination curves are a result of averaging all the tests from the two sets, it is logical that these curves seem too conservative in their estimation of allowable storage time, when compared with data from set one alone. The less conservative linear curves help to make them appear closer to the results of the first set.

The hypothesis regarding using the germination Eq. (4.1) to calculate germination as the temperature decreased in the second set of tests did not yield the expected results. This is understandable because Eq. (4.1) is based on data from both sets. In comparing the experimental data from the second set to the results from Eq. (4.1) therefore, the data from the second set appear skewed. Neither set however, is more valid than the other so they can not be used alone to validate the curves. With this in mind, the procedure proposed in Section 4.2.3 on germination predictions using Eq. (4.1), may still be proven valid with further testing. As it is, I could gain no foreseeable advantage from further analysis of the existing



Figure 5.5. Tests with similar temperature schedules at varying storage times.

data alone. Nevertheless, I have made some observations on the data from the two sets. Using two tests with similar temperature schedules (Tests 1-7 and 2-3), I have compared them to the results from the constant temperature tests (Fig. 5.5). Both tests started at 35°C but Test 1-7 was decreased to 20°C after 4 d. This test resulted in nearly identical results as the test that remained at 20°C throughout. Test 2-3 that began at 35°C, was decreased to 20°C after 21 d. Although the drop in temperature may have prevented any further deterioration from this point on, it is scarcely different from the test that remained continually at 35°C. These results are typical of all the tests held at a high temperature for a short term in set one, and the tests held at a high temperature for a long term in set two. This information can provide some cooling boundaries for farmers (Table 5.3). The results are interesting because if cooling does not occur at these times deterioration will result shortly thereafter. This suggests that decay can set in quite suddenly. I have plotted these times on their respective curves (Fig. 5.4). They lie right at the point when the curves begin to turn downward. Based on my results for 35, 30, and 25°C I postulate that cooling from initial grain temperatures of 20°C and 15°C to subzero temperatures as late as roughly 4 wk and 8 wk into storage respectively, may be similarly effective in delaying deterioration of wet grain. Cooling of the grain from any temperature to below -8°C should halt deterioration, as this is the minimum temperature at which psychrophilic organisms (which includes most

Table 5.3.	Length of storage at a given temperature after which
	cooling is still effective in delaying deterioration.

Storage temperature (°C)	Storage time (d)	
35	4	
30	7	
25	14	

Penicillium spp.) can grow (Wallace 1973).

5.3 Microflora

The appearance of visible mould about 1 wk after the germination decreases to 90% does not preclude it from being a useful indicator. The limit of 90% germination is an artificially derived one. The actual end use of the grain will determine the usefulness of this parameter. If the farmer is to use the wheat personally, say for animal feed, a high germination need not be maintained. The delay by about 1 wk to learn that it is beginning to spoil may still be useful to a farmer in showing that the storage conditions need to be changed to prevent further deterioration. Moreover, a germination check is not a practical procedure for a farmer. While checking for visible mould is easy, especially because it usually first appears on the surface of the grain bulk.

Although I identified many species of microflora to be living in the wheat throughout this study, the predominant species were as expected. The interrelationships among these species of fungi and between these fungi and several common deterioration parameters have been studied. Researchers have done tests similar to the ones in this study and have expressed the relationships in correlation matrices. I have presented some correlation coefficients from these matrices in Table 5.4. Values approaching +1.0 indicate that the variables increase or decrease similarly, a strong positive correlation. Values approaching -1.0 indicate that as one variable increases the other decreases, a strong negative correlation. Values that remain ca. 0.0 indicate minimal correlation between the variables. The findings listed below support the activities that occurred here. The data show that there is no correlation between *A. glaucus* gr. and the FAV. A negative correlation exists however between *A. glaucus* gr. and germination, supporting the decrease illustrated when *A. glaucus* gr. rose

to around 60%. The data further indicate that *Alternaria* spp. and *Penicillium* spp. are highly negatively correlated, hence the decrease in *Alternaria* spp. as the *Penicillium* spp. began to dramatically increase. This is especially apparent in the second set of tests where *Penicillium* spp. were more evident. Finally, these correlations show that *Penicillium* spp. are strongly correlated to an increase in the FAV. Similarly Bottomley et al. (1952) found that FAV increases in corn were more pronounced in the presence of *Penicillium* spp. than *A. glaucus* gr. This would suggest that FAV should be noticeably higher in the second set of tests than in the first, due to the increased occurrence of *Penicillium* spp. in the second set. A review of the data validates this supposition.

Table 5.4. ^T Correlation coefficient	s for	microflora,	germination,	and F	FAV.
-------------------------------------------------	-------	-------------	--------------	-------	------

	A. glaucus gr.	Penicillium spp.	germination	FAV
Alternaria spp.	- 0.152	- 0.492 [- 0.884]	+0.646 [+0.841]	X‡
<i>A. glaucus</i> gr.		+ 0.052	- 0.367	+ 0.047
Penicillium spp.			- 0.642 [-0.887]	+ 0.620

Sources: Wallace et al. (1983); [Wallace and Sinha (1962)]

[‡] X indicates no correlation listed in the literature.

5.4 Fat acidity value

The sigmoidal pattern of the germination-time curves is seen again in the FAV-time curves (Section 4.3.2, Fig. 4.7). The reasons for this are more intricate than the mere depletion of nutrient reserves. Once nutrient reserves begin to be depleted, some fungi will consume portions of the free fatty acids after they have produced them. As well, when the abiotic conditions change, different groups of moulds may succeed those originally present, as indicated above in the replacement of *Alternaria* spp. with the *Penicillium* spp. The amount of free fatty acids (FFA) produced varies with species of fungus, and very probably

with strains within a species (Christensen and Kaufmann 1969). Assuming that after an increase these conditions may cause a decrease, or at the very least a plateau in the quantity of FFA present, is therefore reasonable.

6. CONCLUSIONS

The conclusions of this study with respect to the stated objectives are:

1. The rate of reduction in the germination of hard red spring wheat stored at 17% moisture content (m.c.) over a period of 3 mo can be modelled with the equation:

$$G = \frac{98}{\left[1 + \left(\frac{T}{c}\right)^{b}\right]^{e}}$$

where: G = germination rate (%)

T =storage time (d); valid for 1-84 d.

b, c, and e are constants that differ for each of the 5 temperatures studied.

2. a) An equation to model the allowable storage time of hard red spring wheat at 17% m.c. and constant temperature conditions is:

$$T = 10^{2.5 - 0.045 t}$$

where: t = temperature (°C); valid between 15 and 35°C.

2. b) The equation listed in part 2a) above can effectively be used with a deterioration index of 1.4 to determine the allowable storage time of fresh grain under decreasing temperature conditions.

3. a) Visible mould generally appears about 5 d after the germination decreases to 90%. It can be a useful and practical indicator of deterioration depending on the end use of the grain.

3. b) The increase in the free fatty acids in wheat, induced by microflora, can be correlated

to the decrease in germination for wheat. If the rate of change of the fat acidity value can be measured, then a 25% increase from its initial value suggests deterioration for wheat at 17% m.c. Changes which may occur in the rate after this point are irrelevant.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

1. The experimental results for wheat stored at decreasing temperatures yielded longer allowable storage times than the results from stringing the constant temperature germination prediction curves together. This unknown factor that is apparently causing the deterioration time to be lengthened from that of the constant temperature grain needs to be determined, so that it can be incorporated into Eq. (4.1).

2. Similar tests to those conducted in this experiment should be repeated with other constant moisture contents to determine how moisture content affects the coefficients in Eq. (4.1) and (4.2).

3. Similar tests to those conducted in this experiment should be repeated with changing moisture contents and constant temperatures.

Data resulting from these recommendations could be combined with the data from this study, for an overall analysis. Conducting tests where both the moisture content and the temperature are changing would be most interesting, but such tests would be extremely difficult to analyse, therefore they have not been recommended here.

8. REFERENCES

- AACC. 1962. Fat acidity general method. Method 02-01A (approved April 1961) in: Cereal laboratory methods, 7th ed. American Association of Cereal Chemists, St. Paul, MN.
- Abramson, D., R.N. Sinha, and J.T. Mills. 1990. Mycotoxin formation in HY-320 wheat during granary storage at 15 and 19% moisture content. Mycopathologia. 111:181-189.
- Agriculture statistics. Manitoba agriculture weekly crop reports. Economics Branch. Manitoba Agriculture. Winnipeg, MB.
- ASAE. 1993. Moisture measurement unground grain and seeds. ASAE S352.2. Page 449 in: ASAE Standards 1993. American Society of Agricultural Engineers, St. Joseph, MI.
- Baker, D., M.H.Neustadt, and L. Zeleny. 1957. Application of the fat acidity test as an index of grain deterioration. Cereal Chemistry 34:226-233.
- Bason, M.L., J.A. Ronalds, and C.W. Wrigley. 1993. Prediction of safe storage life for sound and weather-damaged malting barley. Cereal Foods World. 38(5): 361-363.
- Bason, M. L., P.W. Gras, and H.J. Banks. 1994. Modelling the effects of temperature, water activity and storage atmosphere on the viability of stored maize and paddy. Pages 677-683 in: Stored product protection. Proceedings of the 6th international working conference on stored-product protection. Vol.II. E. Highley, E.J. Wright, H.J. Banks, and B.R. Champ (ed.). CAB International. Canberra, Australia.
- Bottomley, R.A., C. M, Christensen, and W.F. Geddes. 1952. Grain storage studies. X. The influence of aeration, time, and moisture content on fat acidity, nonreducing sugars, and mold flora of stored yellow corn. Cereal Chemistry. 29:53-64.
- Brook, R.C. 1987. Modelling grain spoilage during near-ambient grain drying. Div. Note DN 1388, AFRC Institute of Engineering Research, Silsoe, Bedford. 20 p.
- Brooker, D.B. and A.K. Duggal. 1982. Allowable storage time of corn as affected by heat buildup, natural convection and aeration. Transactions of the ASAE (American Society of Agricultural Engineers). 25:808-810.
- Canadian Grain Commission. 1991. Official grain grading guide: 1991 edition. Winnipeg, MB.
- Christensen, C.M. and H.H. Kaufmann. 1969. Grain storage; the role of fungi in quality loss. University of Minnesota press, Minneapolis, MN. 153 p.
- Demianyk, C.J. 1995. Personal Communication on 1995 07 24 with C.J. Demianyk, Stored product entomologist, Cereal Research Centre, Agriculture and Agri-food Canada, Winnipeg, MB., Canada.

- Donald, W.W. and C.J. Mirocha. 1977. Chitin as a measure of fungal growth in stored corn and soybean seed. Cereal Chemistry. 54(3): 466-474.
- Dorworth, C.E. and C.M. Christensen. 1968. Influence of moisture content, temperature, and storage time upon changes in fungus flora, germinability, and fat acidity values of soybeans. Phytopathology. 58:1457-1459.
- Ellis, R.H. and E.H. Roberts. 1980. Improved equations for the prediction of seed longevity. Annals of Botany. 45:13-30.
- Environment Canada. Atmospheric environment service. Downsview, ON.
- Fernandez, A., R. Stroshine, and J. Tuite. 1982. Carbon dioxide production and mold growth in stored corn. Paper 82-3016. American Society of Agricultural Engineers, St. Joseph, MI. 32 p.
- Fraser, B.M. 1979. Solar grain drying in Canada: a simulation study. Unpublished M.Sc. thesis, Department of Agricultural Engineering, University of Manitoba. Winnipeg, MB. 175p.
- Friday, D.C., R.L. Stroshine, and J.Tuite. 1986. Improving low temperature drying using mold resistant hybrids. ASAE paper no. 86-3010. American Society of Agricultural Engineers. St. Joseph, MI. 18 p.
- Golubchuk, M., H. Sorger-Domenigg, L.S. Cuendet, C.M. Christensen, and W.F. Geddes. 1956. Grain storage studies. XIX. Influence of mold infestation and temperature on the deterioration of wheat during storage at approximately 12% moisture. Cereal Chemistry 33: 45-52.
- Golobchuk, M., L.S. Cuendet, and W.F. Geddes. 1960. Grain storage studies. XXX. Chitin content of wheat as an index of mold contamination and wheat deterioration. Cereal Chemistry. 37:405-411.
- Hamer, A., J. Lacey, and N. Magan. 1991. Use of an automatic electrolytic respirometer to study respiration of stored grain. Pages 321-329 in: Proceedings 5th international working conference on stored-product protection. Vol.I. F.Fleurat-Lessard and P. Ducom. (ed.). France.
- Harrington, J.F. 1963. Practical advice and instructions on seed Storage. Proceedings of the International Seed Testing Association 28: 989-94.
- Juliano, B.O. 1994. Concerns for quality maintenance during storage of cereals and cereal products. Pages 663-665 in: Stored product protection. Proceedings of the 6th international working conference on stored-product protection. Vol. 2. E. Highley, E.J. Wright, H.J. Banks, and B.R. Champ (ed.). CAB International. Canberra, Australia.
- Kreyger, J. 1972. Drying and storing grains, seeds and pulses in temperate climates. Institute for storage and processing of agricultural products. Wageningen, Holland.

- Lacey, J., A. Hamer, and N. Magan. 1994. Respiration and losses in stored wheat under different environmental conditions. Pages 1007-1013 in: Stored Product Protection. Proceedings of the 6th international working conference on storedproduct protection. Vol.II. E. Highley, E.J. Wright, H.J. Banks and B.R. Champ. (ed.). CAB International. Canberra, Australia,
- Mann, D.D. 1995. Development of a grain storage information system for Canadian farmers and grain storage managers. Unpublished M.Sc. Thesis, Department of Agricultural Engineering, University of Manitoba. Winnipeg, MB. 88p.
- Marfleet, I., N. Magan, and J. Lacey. 1991. The relationship between fungal biomass, ergosterol and grain spoilage. Pages 405-411 in: Proceedings 5th international working conference on stored-product protection. Vol. I. F. Fleurat-Lessard & P. Ducom (ed.). France.
- Metzger, J.F. and W.E. Muir. 1983. Computer model of two-dimensional conduction and forced convection in stored grain. Canadian Agricultural Engineering 25:119-125.
- Mills, J.T. 1992. Safe storage guidelines for grains and their Products. Postharvest News and Information. Vol. 3(6): 111N-115N.
- Mills, J.T. and H.A.H. Wallace. 1979. Microflora and condition of cereal seeds after a wet harvest. Canadian Journal of Plant Science. 59:645-651.
- Mills, J.T. and W.K. Kim. 1977. Chemical and physiological characteristics of heatdamaged stored Rapeseed. Canadian Journal of Plant Science. 57:375-381.
- Mills, J.T., R.N. Sinha, and H.A.H. Wallace. 1978. Multivariate evaluation and isolation techniques for fungi associated with stored rapeseed. Phytopathology 68:1520-1525.
- Milner, M. and W.F. Geddes. 1954. Respiration and heating. Pages152-219 in: Storage of cereal grains and their products. J.A. Anderson and A.W. Alcock (Ed.). American Association of Cereal Chemists. St. Paul, MN.
- Nandi, B. 1978. Glucosamine analysis of fungus-infected wheat as a method to determine the effect of antifungal compounds in grain preservation. Cereal Chemistry. 55(2): 121-126.
- Pomeranz, Y. 1992. Biochemical, functional, and nutritive changes during storage. Pages 55-141 in: Storage of cereal grains and their products. D.B. Sauer (Ed.). American Association of Cereal Chemists, inc. St. Paul, MN.
- Prasad, D.C., W.E. Muir, and H.A.H. Wallace. 1978. Characteristics of freshly-harvested wheat and rapeseed. Transactions of the ASAE (American Society of Agricultural Engineers). 21: 782-784.
- Roberts, E.H. and F.H. Abdalla. 1968. The influence of temperature, moisture, and oxygen on period of seed viability in barley, broad beans, and peas. Annals of Botany. 32:97-117.

- Sanderson, D.B., W.E. Muir, R.N. Sinha, D. Tuma, and C.I. Kitson. 1989. Evaluation of a model of drying and deterioration of stored wheat at near-ambient conditions. Journal of Agricultural Engineering Research. 42:219-233.
- Sauer, D.B., R.A. Meronuck, and C.M. Christensen. 1992. Microflora. Pages 313-340 in: Storage of cereal grains and their products. D.B. Sauer (ed.). American Association of Cereal Chemists, Inc. St. Paul, MN.
- Saul, R.A. and J.L. Steele. 1966. Why damaged shelled corn costs more to dry. Agricultural Engineering 47(6): 326-329.
- Seitz, L.M., D.B. Sauer, H.E. Mohr, and D.F. Aldis. 1982a. Fungal growth and dry matter loss during bin storage of high-moisture corn. Cereal Chemistry 59(1): 9-13.
- Seitz, L.M. D.B. Sauer and H.E. Mohr. 1982b. Storage of high-moisture corn: fungal growth and dry matter loss. Cereal Chemistry 59(2): 100-105.
- Seitz, L.M., H.E. Mohr, R. Burroughs, and D.B. Sauer. 1977. Ergosterol as an indicator of fungal invasion in grains. Cereal Chemistry. 54(6): 1207-1217.
- Semeniuk, G. 1954. Microflora. Pages 77-151. in: Storage of cereal grains and their products. J.A. Anderson and A.W. Alcock (ed.). American Association of Cereal Chemists. St. Paul, MN.
- Siegenthaler, P.A. and V. Douet-Orhant. 1994. Relationship between the ATP content measured at three imbibition times and germination of onion seeds during storage at 3, 15, and 30°C. Journal of Experimental Botany. 45(279): 1365-1371.
- SigmaPlot. 1988. SigmaPlot scientific graph system: user's manual. Jandel Scientific, Corte Madera, CA.
- Sinha, R.N. 1983. Effects of stored-product beetle infestation on fat acidity, seed germination, and microflora of wheat. Journal of Economic Entomology. 76(4): 813-817.
- Sinha, R. N., J.T. Mills, H.A.H. Wallace, and W.E. Muir. 1981. Quality assessment of rapeseed stored in ventilated and non-ventilated farm bins. Sciences des Aliments. 1(2): 247-263.
- Sinicio, R. 1994. Computer simulation of aerated wheat stored in tropical and subtropical climates. Unpublished Ph.D. thesis, Department of Agricultural Engineering, University of Manitoba. Winnipeg, MB. 209 p.
- Sorger-Domenigg, H., L.S. Cuendet, C.M. Christensen, and W.F. Geddes. 1955. Grain storage studies. XVII. Effect of mold growth during temporary exposure of wheat to high moisture contents upon the development of germ damage and other indices of deterioration during subsequent storage. Cereal Chemistry. 32:270-285.

STATISTICA 5.0. StatSoft, Tulsa, Okla.

- Steele, J.L., R.A. Saul, and W.V. Hukill. 1969. Deterioration of shelled corn as measured by carbon dioxide production. Transactions of the ASAE (American Society of Agricultural Engineers) 12: 685-689.
- Stroshine, R.L. A.W. Kirleis, J.F. Tuite, L.F. Baumann, and A. Emam. 1986. Differences in grain quality among selected corn hybrids. Cereal Foods World. 31(4): 311-316.
- Thompson, T.L. 1972. Temporary storage of high-moisture shelled corn using continuous aeration. Transactions of the ASAE (American Society of Agricultural Engineers). 15(2): 333-337.
- Trisvyatskii, L.A. 1969. Storage of Grain. Vol. 2. D.M. Keane (translation from Russian). N.L. Kent and J.A. Freeman (ed.). National Lending Library for Science and Technology, Boston Spa, Yorkshire, England. 245-532 p.
- Wallace, H.A.H. 1973. Fungi and other organisms associated with stored grain. Pages 71-98 in: Grain storage: part of a system. R.N. Sinha and W.E. Muir (ed.). The AVI publishing company, inc. Westport, Connecticut.
- Wallace, H.A.H. and R.N. Sinha. 1962. Fungi associated with hot spots in farm-stored grain. Canadian Journal of Plant Science. 42:130-141.
- Wallace, H.A.H., P.L. Sholberg, R.N. Sinha, and W.E. Muir. 1983. Biological, physical and chemical changes in stored wheat. Mycopathologia. 82:65-76.
- White, N.D.G., R.N. Sinha, and W.E. Muir. 1982. Intergranular carbon dioxide as an indicator of biological activity associated with the spoilage of stored Wheat. Canadian Agricultural Engineering. 24(1): 35-42.
- Wilcke, W.F., H.F. Ng, R.A. Meronuck, J.P. Lang, and R.V. Morey. 1995. Dry matter loss in storage for sound and diseased wheat. Paper 95-6132. American Society of Agricultural Engineers, St. Joseph, MI. 10 p.
- Wu, Lung-Chi and M.A. Stahmann. 1975. Chromatographic estimation of fungal mass in plant materials. Phytopathology. 65:1032-1034.
- Zeleny, L. 1954.Chemical, physical, and nutritive changes during storage. Pages 46-76 in: Storage of cereal grains and their products. J.A. Anderson and A.W. Alcock (ed.). American Association of Cereal Chemists. St. Paul, MN.

APPENDIX A: Germination data.

					St	orage d	ay			
Test	Replicate	0	4	7	21	35	49	63	77	84
1	а	96	100	100	76	74	6	2	0	0
	a	98 98	94 98	94 04	92 00	76	0	0	0	0
2	0	00	00	9 4	90	90	0	0	0	0
2	a b	98 98	92 94	88 98	92 96	94 88	62 36	14 1	2	12
	c	96	96	98	98	96	48	- - 16	10	8
3	а	98	100	98	96	94	78	36	24	26
	b	94	92	96	86	98	84	66	48	36
	С	100	94	94	96	82	78	46	24	22
4	a	98	96	94	96	96	84	80	36	42
	a C	98	98 80	100 Q4	98 94	94 96	88	42	26 58	26 54
E	0	00	00	04	07	30	00	90	50	54
5	a h	96 100	92 94	92 08	96	100	88	96 02	88	62 59
	c	96	96	98	100	96	94 92	92 94	80 80	50 66
6	а	98	100	100	100	100	92	38	18	18
	b	96	96	88	88	96	86	32	18	20
	С	98	100	94	96	96	66	28	14	18
7	а	100	82	92	94	96	92	76	78	70
	b	96	92	90	96	98	96	78	58	46
	С	100	86	86	96	98	92	74	16	26
8	a	96	74	96	98	92	74	44	32	12
	α 2	96 94	98	96	100 08	94	84	62 50	32	2
0	0	04	400	00	90	90	90	50	.34	0
9	a b	94 98	96	90	98	86	94	96	90	78
	c	96	90	90	90 92	92 94	90 92	82	76 94	80 74
10	а	86	90	90	96	96	<u>ол</u>	62	36	12
	b	88	90	90	94	94	84	68	58	4
	С	94	92	92	98	94	90	46	26	0
11	а	98	88	88	96	92	86	78	42	30
	b	98	82	82	98	98	82	68	48	40
	С	90	88	88	90	94	88	80	58	60
12	a	96	86	86	96	84	98	96	76	48
	b	96 04	90	90	88	96	90	88	64	60
	<u>ل</u>	34	04	04	00	90	70	90	90	66

Table A.1. Germination (%) in set 1.

First record of germination remaining below 90%.
						Storag	e day			
Test	Replicate	0	7	14	21	35	49	63	77	91
1	a	94	88	34	22	4	2	0	0	0
	b	92	100	86	24	4	0	0	n.d.	n.d.
	c	98	98	62	18	4	0	0	0	0
2	a	100	94	82	36	14	28	12	10	20
	b	96	98	94	26	22	14	16	12	14
	c	92	96	78	22	16	8	12	6	6
3	a	100	88	28	14	12	12	20	10	14
	b	96	94	78	22	14	18	12	16	16
	c	100	56	62	22	16	14	16	10	20
4	a	100	90	36	38	20	12	18	16	14
	b	92	82	74	22	20	12	16	14	20
	c	92	72	96	62	18	16	12	24	12
5	a	100	n.d.	64	52	20	14	16	16	8
	b	98	n.d.	100	86	20	16	14	18	12
	c	96	n.d.	100	48	14	18	10	24	18
6	a	96	n.d.	98	82	18	22	18	22	30
	b	96	n.d.	98	78	28	22	28	20	30
	c	98	n.d.	96	50	24	12	24	28	20
7	a	98	92	98	82	24	12	14	16	20
	b	96	100	60	68	26	8	18	20	20
	c	98	98	78	64	36	16	14	24	16
8	a	98	n.d.	90	74	36	24	20	18	24
	b	98	n.d.	100	86	30	12	16	18	6
	c	98	n.d.	92	42	18	16	14	12	16
9	a	100	n.d.	94	50	16	18	10	22	20
	b	100	n.d.	98	68	26	16	16	22	14
	c	98	n.d.	80	46	22	24	22	24	26
10	a	94	n.d.	94	62	34	24	26	20	26
	b	100	n.d.	98	80	24	30	20	26	24
	c	96	n.d.	100	44	44	24	12	18	18
11	a	92	n.d.	98	78	46	14	22	22	12
	b	98	n.d.	96	60	24	22	26	12	10
	c	98	n.d.	86	76	38	16	18	22	26
12	a	98	88	44	22	8	22	24	28	14
	b	98	90	90	44	34	18	14	14	12
	c	98	98	70	68	42	16	20	16	18

Table A.2. Germination (%) in set 2.

First record of germination remaining below 90%.

n.d. Indicates no data.

的感觉感到

				St	orage d	ay			
Temperature	0	7	14	21	35	49	63	77	84
35°C	96	89	68	31	4	1	0	0	0
	(3.2)	(11.6)	(22.3)	(16)	(0)	(1)	(0)	(0)	(0)
30°C	96	95	90	65	25	18	11	7	7
	(3.4)	(3.7)	(13.2)	(15.9)	(6.5)	(6.4)	(6.4)	(4.6)	(7.2)
25°C	97	96	95	75	64	54	49	32	28
	(2.8)	(2)	(5)	(17.3)	(31.6)	(23.5)	(15.3)	(13.9)	(7.2)
20°C	95	91	94	95	95	87	67	43	28
	(4)	(5.9)	(4.8)	(3.7)	(3.2)	(5.5)	(15.1)	(16.6)	(23.1)
15°C	97 (2.3)	96 (3.5)	-	99 (2.3)	97 (2.3)	91 (3.1)	94 (2)	83 (4.6)	62 (4)

 Table A.3.
 Combined mean germination data for each temperature studied.

() = one standard deviation

APPENDIX B: Visible mould data.

Index	Definition
0	No visible mould growth
1	Visible mould on few kernels at peripherals of bags
2	Visible mould throughout the bulk of the sample
3	Sample beginning to turn grey or dull in colour
4	Sample dull or grey in colour
5	Sample extensively damaged

.

								Sto	orage	day					
Test	Replicate	0	4	7	14	21	28	35	42	49	56	63	70	77	84
1	a	0	0	0	0	0	0	0	1	2	4	4	5	5	5
	b	0	0	0	0	0	0	0	1	2	4	4	5	5	5
	c	0	0	0	0	0	0	0	1	2	4	4	5	5	5
2	a	0	0	0	0	0	0	0	1	2	2	4	4	4	4
	b	0	0	0	0	0	0	0	1	1	2	4	4	4	4
	c	0	0	0	0	0	0	0	1	1	2	4	4	4	4
3	a	0	0	0	0	0	0	0	1	1	1	2	2	2	3
	b	0	0	0	0	0	0	0	1	1	1	1	1	2	2
	c	0	0	0	0	0	0	0	1	1	2	2	2	2	2
4	a	0	0	0	0	0	0	0	0	1	1	2	2	2	3
	b	0	0	0	0	0	0	0	0	1	2	2	2	2	2
	c	0	0	0	0	0	0	0	0	0	1	1	1	2	2
5	a	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	b	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	c	0	0	0	0	0	0	0	0	0	0	0	0	1	1
6	a	0	0	0	0	0	0	0	· 0	1	2	2	2	2	3
	b	0	0	0	0	0	0	0	0	1	2	2	2	2	3
	c	0	0	0	0	0	0	0	1	2	2	3	3	3	4
7	a	0	0	0	0	0	0	0	0	0	1	1	1	2	2
	b	0	0	0	0	0	0	0	0	1	1	2	2	2	3
	c	0	0	0	0	0	0	0	1	1	2	2	3	3	4
8	a	0	0	0	0	0	0	0	1	2	2	2	2	2	2
	b	0	0	0	0	0	0	0	1	1	2	2	2	2	2
	c	0	0	0	0	0	0	0	1	1	2	2	3	3	3
9	a	0	0	0	0	0	0	0	0	0	0	0	0	1	1
	b	0	0	0	0	0	0	0	0	0	0	1	0	1	1
	c	0	0	0	0	0	0	0	0	0	0	1	0	0	1
10	a	0	0	0	0	0	0	0	0	0	2	3	2	2	2
	b	0	0	0	0	0	0	0	1	1	2	2	2	2	2
	c	0	0	0	0	0	0	0	1	1	2	2	3	3	4
11	a	0	0	0	0	0	0	0	1	1	1	3	3	3	3
	b	0	0	0	0	0	0	0	1	1	1	3	3	3	3
	c	0	0	0	0	0	0	0	1	1	1	2	2	3	3
12	a	0	0	0	0	0	0	0	0	0	1	1	1	1	2
	b	0	0	0	0	0	0	0	0	1	1	1	1	1	2
	c	0	0	0	0	0	0	0	0	1	0	1	1	1	2

 Table B.1.
 Level of visible mould apparent in set 1.

First day when visible mould appears.

								Sto	rage	day					
Test	Replicate	0	7	14	21	28	35	42	49	56	63	70	77	84	91
1	a	0	0	3	4	4	4	4	4	4	4	4	4	5	5
	b	0	0	1	4	5	5	5	5	6	6	6	6	6	6
	c	0	0	3	4	4	5	4	4	5	5	5	5	5	6
2	a	0	0	1	3	4	4	4	4	4	4	4	5	4	4
	b	0	0	1	3	4	4	4	4	4	4	5	5	5	5
	c	0	0	2	3	5	4	4	4	4	4	4	4	4	4
3	a	0	0	3	4	4	4	4	4	4	4	4	4	4	4
	b	0	0	1	4	4	4	4	4	4	4	4	4	4	4
	c	0	0	1	4	4	4	4	4	4	4	4	4	5	4
4	a	0	0	3	4	3	4	4	4	4	4	4	4	4	4
	b	0	0	2	4	3	4	4	4	4	4	4	4	4	4
	c	0	0	1	2	4	4	4	4	4	4	4	4	5	4
5	a	0	0	1	2	3	5	5	5	5	5	5	5	5	5
	b	0	0	0	1	3	5	5	5	5	5	5	5	5	5
	c	0	0	0	2	3	4	4	4	4	4	4	5	4	4
6	a	0	0	0	1	4	3	3	3	3	4	4	3	4	3
	b	0	0	0	1	3	3	3	3	3	4	4	4	4	3
	c	0	0	0	2	4	3	3	3	3	4	4	4	4	4
7	a	0	0	0	1	3	3	3	4	4	4	4	4	4	4
	b	0	0	2	2	3	3	4	4	4	4	4	4	4	4
	c	0	0	1	1	3	3	3	3	3	3	4	4	4	4
8	a	0	0	1	1	2	4	4	4	4	4	4	4	4	4
	b	0	0	0	1	3	5	5	5	5	4	4	4	4	4
	c	0	0	1	2	4	4	4	4	4	4	4	4	4	4
9	a	0	0	0	2	4	4	4	4	4	4	4	4	4	4
	b	0	0	0	1	3	3	3	3	3	3	3	3	3	3
	c	0	0	0	1	3	3	3	3	3	3	3	3	3	3
10	a	0	0	0	1	2	4	5	5	4	4	4	3	3	3
	b	0	0	0	1	3	4	4	4	4	4	4	3	3	3
	c	0	0	0	2	3	4	5	4	4	4	4	3	3	3
11	a	0	0	0	1	2	3	5	5	5	5	5	5	5	5
	b	0	0	0	2	3	4	4	4	4	4	4	4	5	5
	c	0	0	0	1	3	3	4	4	4	4	4	4	5	5
12	a	0	0	3	3	3	3	4	4	4	4	4	4	4	4
	b	0	0	0	3	3	3	4	4	4	4	4	4	5	4
	c	0	0	0	1	2	3	4	4	4	5	5	5	5	5

 Table B.2.
 Level of visible mould apparent in set 2.

First day when visible mould appears.

APPENDIX C: Microflora data using the salted filter paper method.

			Mi	croflora % re	adings				
Day	Replicate	Alt	A.gl A.	ochr Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	92 64 76	2						
4	a b c	68 70 64	2 4 16	2		4			
7	a b c	42 56 46	72 56 76						
21	a b c	34 22 44	90 88 92	2					
35	a b c	14 6 6	100 98 96						
49	a b c	4	86 98 90					28 2 14	
63	a b c		86 74 58					94 84 96	
77	a b c		26 28 28 38 2	B 3 2				46 94 92	
84	a b c		24 8 14 2	2				40 88 94	

 Table C.1. Microflora reading on salted filter paper for test 1-1.

				Microflo	ra % re	adings	;			
_Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	а	76								
	b	66								
	С	66								
4	а	76								
	b	68	2							
	С	76	4					2		
7	а	60	10							
	b	68	38							
	С	60	32							
21	а	68	68							
	b	64	68							
	С	72	56							
35	а	40	88							
	b	34	94							
	С	30	74							
49	а	26	84						2	
	b	32	80							
	С	34	88							
63	а	6	58				2		80	
	b	4	44						96	
	С		76						58	
77	а		22	6					98	
	b		12	4					98	
	С		30	6					92	
84	а	4	32	2					82	
	b		14	2					96	
	С	4	22						98	

Table C.2. Microflora reading on salted filter paper for test 1-2.

			Microflo	ra % re	adings	;			
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	68 74 80							
4	a b c	72 74 84			2				
7	a b c	60 78 62	16						
21	a b c	54 60 72	66 62 50						
35	a b c	46 32 38	68 76 48						
49	a b c	58 46 46	66 66 70					2 2 2	
63	a b c	20 30 16	90 88 78						
77	a b c	14 30 6	90 90 94					4 2 36	
84	a b c	22 34 16	86 92 96					10 4 54	

Table C.3. Microflora reading on salted filter paper for test 1-3.

				Microflo	ora % re	adings	;			
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	66 58 88								
4	a b c	58 66 76			2	2				
7	a b c	76 76 68	4							
21	a b c	68 74 76	20 24 12		2	2				
35	a b c	48 50 78	56 46 16							
49	a b c	66 64 72	44 64 48							
63	a b c	32 18 20	78 86 70							
77	a b c	38 16 36	70 96 66						4	
84	a b c	48 46 58	86 90 66						2 4 4	

Table C.4. Microflora reading on salted filter paper for test 1-4.

.

			Microflo	ora % re	adings				
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	82 70 76							
4	a b c	72 78 56		2 2	2				
7	a b c	76 76 68	4						
21	a b c	74 78 62	2 4						
35	a b c	68 70 66	4 6 6						
49	a b c	58 64 78	12 24 14						
63	a b c	58 52 42	52 50 54			2			
77	a b c	44 50 60	66 82 44					2 10 6	
84	a b c	52 60 64	62 58 60					4 4 8	

Table C.5. Microflora reading on salted filter paper for test 1-5.

	Microflo	ora % re	adings				
A.gl	A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
4		2					
8		-					
10							
36							
58							
56							

Table C.6. Microflora reading on salted filter paper for test 1-6.

Alt

Replicate

а b

С

а

b

С

а b

С

а

b

С

а

b

С

а

b

С

а b С

а

b

С

а

b

С

Day

40 32	68 86			
4 20 10	92 90 92			
6 6	70 84 98			
18 18 4	74 28 94	****	 	

				Microflo	ra % re	eadings	;			······
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	62 58 76								
4	a b c	80 54 66	2 2		6	2				
7	a b c	72 62 74	6 6							
21	a b c	64 66 56	56 52 66			2				
35	a b c	60 54 54	62 64 74							
49	a b c	62 48 44	44 60 60							
63	a b c	34 26 14	90 86 90						4	
77	a b c	20 26 8	58 94 90							
84	a b c	36 34 8	84 100 94						10 8	

Table C.7. Microflora reading on salted filter paper for test 1-7.

				Microflo	ora % re	adings				
Day	Replicate	Alt	A.gl	A.ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	82 72 68								
4	a b c	86 68 58			30					
7	a b c	80 66 56	8 2		28					
21	a b c	64 54 72	46 62 44		2					
35	a b c	50 60 66	66 46 52							
49	a b c	24 30 66	86 74 46							
63	a b c	16 28 36	96 86 90	8					10 12	
77	a b c	8 4 6	88 92 90	4						
84	a b c		100 100 100						72 42 14	

Table C.8. Microflora reading on salted filter paper for test 1-8.

	Microflo	ra % re	adings	;			
A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
		4					
32							

Fable C.9.	Microflora	reading	on salted	filter	paper	for	test 1	1-9.
------------	------------	---------	-----------	--------	-------	-----	--------	------

Alt

68 66

Replicate

a b

Day 0

	С	84			
4	a b c	74 78 72		4	
7	a b c	66 66 82	32 24 50		
21	a b c	44 56 42	50 62 58		
35	a b c	54 56 58	44 40 60		
49	a b c	62 68 62	46 26 50		
63	a b c	42 40 32	68 62 64		
77	a b c	30 38 24	60 58 76		
84	a b c	60 58 36	58 78 72		2

				Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	74 66 82								
4	a b c	62 74 58	2 10							
7	a b c	68 70 54	56							
21	a b c	46 48 60	44 62 56							
35	a b c	68 82 66	50 58 70							
49	a b c	34 50 50	54 56 66							
63	a b c	38 38 28	68 78 76						2 2	
77	a b c	14 18 12	44 82 98	2						
84	a b c		100 100 100						38 10 20	76 76 76

Table C.10. Microflora reading on salted filter paper for test 1-10.

1			Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl A.ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	74 68 78							
4	a b c	64 72 58							
7	a b c	76 72 64	6						
21	a b c	72 62 58	48 60 38						
35	a b c	64 62 66	54 64 44						
49	a b c	52 44 42	42 52 46						
63	a b c	26 32 26	82 68 58						
77	a b c	22 16 16	94 88 72						
84	a b c	18 30 40	86 92 82					12 10 8	

 Table C.11. Microflora reading on salted filter paper for test 1-11.

		Microflora % readings								
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	68 66 76								
4	a b c	60 74 60	2		2					
7	a b c	74 68 64								
21	a b c	64 60 58	20 14 30							
35	a b c	70 78 76	42 30 38							
49	a b c	52 48 52	40 26 54							
63	a b c	52 22 38	48 62 66						4	
77	a b c	22 30 32	82 88 60						2 2	
84	a b c	52 34 28	74 74 64						10 8 2	

 Table C.12.
 Microflora reading on salted filter paper for test 1-12.

				Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	64 64 60	10 2							
14	a b c	14 26 28	92 92 96						4 4	
21	a b c	22 24 24	86 96 96						74 80 38	
35	a b c	6 2	62 42 12	6 4 20					94 86 94	
49	a b c		46 92 46	66 24 16					96 94 100	
63	a b c	2	78 92 22	12 4 6				6	90 100 94	
77	a b c		82 n.d. 26						58 n.d. 80	4 n.d. 64
91	a b c		26 n.d. 30						92 n.d. 92	

Table C.13. Microflora reading on salted filter paper for test 2-1.

n.d. Indicates no data

				Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b	56 58								
	С	58								
14	a b	18 24	92 92						2	
	C	20	90						2	
21	a b c	24 10 10	98 100 94						78 68 72	
35	a b c	10 4 10	48 36 42						86 92 80	
49	a b c		10 26 18	6					100 94 96	
63	a b c	6 6 6	12 20 12	2 18					96 94 96	
77	a b c	2	16 26 22						94 86 78	2 32
91	a b c	8 2 10	32 42 14						88 94 94	22

Table C.14. Microflora reading on salted filter paper for test 2-2.

-

.

.

			Ν	Microflo	ora % re	adings	;			
Day	Replicate	Alt	A.gl A	4.ochr	Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b	50 54								
14	c a b	58 18 18	94 80			4				
21	a b c	4 16 14	96 96 98						46 76 40	
35	a b c	2 16 16	82 84 68						64 80 86	
49	a b c	6 2 4	68 28 16	14 6 12					94 100 98	2
63	a b c	4	72 28 36						100 98 100	
77	a b c	2	72 52 32						92 98 92	
91	a b c	2	64 36 32						98 100 100	

Table C.15. Microflora reading on salted filter paper for test 2-3.

	,			Microfic	ra % re	adings		
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc Pen Rhiz
0	a b c	54 48 54						
14	a b c	26 28 20	86 98 84					72 4 2
21	a b c	14 8 26	96 100 88	·			2	92 48 16
35 [.]	a b c	16 4	50 34 58					92 96 98
49	a b c	2	10 10 2	10 12 6				86 92 100
63	a b c	10 2	32 60 24					96 94 100
77	a b c	14 12	40 50 24					92 92 92
91	a b∝ c	18 6 6	46 38 28					84 4 98 98

Table C.16. Microflora reading on salted filter paper for test 2-4.

			Microflo	ora % re	adings			
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Muc Pen	Rhiz
0	a b c	52 40 50	2					
14	a b c	24 56 40	80 56 74				2	
21	a b c	44 60 40	100 98 90				6 4 40	
35	a b c	12 30 18	50 40 34				84 94 84	
49	a b c	4 10 20	58 36 44				82 82 78	
63	a b c	6 8 8	62 40 42				78 90 86	
77	a b c	2 4 8	56 46 44				82 88 96	
91	a b c	8 10 6	70 46 42				82 76 74	2

 Table C.17. Microflora reading on salted filter paper for test 2-5.

				Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	а	38								
	'n	58								
	С	50								
14	а	64	66							
	b	40	62							
	С	24	90						4	
21	а	-28	72						4	
	þ	48	74						8	
	С	20	88						20	
35	а	32	90						74	
	b	28	94						78	
	С	18	82						92	
49	а	18	96					2	68	
	b	22	80						80	
	С	20	58	2					94	
63	а	50	70						72	
	b	32	88						66	
	С	20	70	2					84	
77	а	28	88						62	
	b	20	82						86	
	С	10	86						82	
91	а	20	100						50	
	b	18	92						70	
	С	14	70						78	

Table C.18. Microflora reading on salted filter paper for test 2-6.

ç

personale

		·	Mi	croflo	ra % re	adings				
Day	Replicate	Alt	A.gl A.d	ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	а	52								
	b	60	2			2				
	С	54	2							
14	а	14	78							
	b	24	90						6	
	С	24	98						8	
21	а	18	74						4	
	b	12	94						т	
	С	6	96							
35	а	24	86						88	
	b	20	96						92	
	С	30	84						32	
49	а	16	56						94	
	b	12	72						100	
	С	14	76						86	
63	а	n.d.	n.d.						nd	
	b	12	60						90	
	С	16	90						74	
77	а	6	36						88	
	b	4	72						94	
	С	18	80						90	
91	а	6	22						96	
	b	16	74						86	
	С	16	78						64	

Table C.19. Microflora reading on salted filter paper for test 2-7.

n.d. Indicates no data

			Mi	croflo	ra % rea	adings				
Day	Replicate	Alt	A.gl A.	ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	72 60 56	2							
14	a b c	30 28 38	72 70 86						6 8	
21	a b c	32 32 30	98 88 88						6 18	
35	a b c	22 26 10	96 88 82						90 94 98	
49	a b c	8 6 2	22 62 46						96 90 96	
63	a b c	2 12	66 60 34						98 90 88	
77	a b c	4 0 6	48 50 32						98 94 96	
91	a b c	6 14 4	76 52 46						94 90 90	

Table C.20. Microflora reading on salted filter paper for test 2-8.

				Microflo	ra % re	adinas				
Day	Replicate	Alt	A.gl	A.ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	58 62 62	2							
14	a b c	16 42 30	84 62 96						6 10 10	
21	a b c	18 38 38	90 96 78						38 16 28	
35	a b c	12 22 14	62 94 100						98 94 94	
49	a b c	6 22 12	54 50 76						98 88 92	
63	a b c	10 20 8	30 48 66						98 84 94	
77	a b c	8 10 4	30 38 84						92 94 92	

abic O.Z.I. micronola reaunity on Sancu Inter paper for lest 2-	Table C.21.	Microflora	reading	on salted	filter	paper	for	test 2-	9.
-----------------------------------------------------------------	-------------	------------	---------	-----------	--------	-------	-----	---------	----

42 90

a b c

				Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	а	60								
	b	60				2				
	С	54	4							
14	а	46	88							
	b	26	74							
	С	22	82							
21	а	46	84						14	
	b	32	66						2	
	С	42	98						8	
35	а	18	94						62	
	b	42	96						36	
	С	28	94						44	
49	а	12	88						26	
	b	24	98						64	
	С	22	86						86	
63	а	10	68						60	
	b	12	90						60	
	С	16	86						74	
77	а	12	86						42	
	b	18	76						60	
	С	20	68	2					50	
91	а	14	92						64	
	b	14	74						44	
	С	20	88						48	

Table C.22. Microflora reading on salted filter paper for test 2-10.

			Mic	roflora % re	eadings				
Day	Replicate	Alt	A.gl A.o	chr Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	54 32 46	2					2	
14	a b c	60 42 56	20 72 36					2	
21	a b c	60 26 45	80 86 84					6 6 4	
35	a b c	26 68 26	60 88 54					38 48 64	
49	a b c	32 26 16	70 82 68				2 2	50 52 62	
63	a b c	22 16 18	84 86 60					36 66 60	
77	a b c	2 16 24	64 88 86					64 44 90	
91	a b c	18 28 36	94 92 84					64 50 52	

 Table C.23. Microflora reading on salted filter paper for test 2-11.

				Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	46 64 44	2							
14	a b c	26 28 22	82 92 76						22 6	
21	a b c	18 24 24	90 94 90	4					74 10	
35	a b c	10 18 18	94 60 78						48 26 8	
49	a b c	8 8 14	46 74 78						76 52 46	
63	a b c	2 10 4	84 84 94						80 54 42	
77	a b c	14 8 22	92 80 82						84 90 60	
91	a b c	6 2 16	90 78 80	2					78 78 54	

Table C.24. Microflora reading on salted filter paper for test 2-12.

APPENDIX D: Microflora data using the filter paper method.

			Mi	icroflor	a % rea	adings				
_ Day	Replicate	Alt	A.gl A.	ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	84 70 74				22 6 8		2		
4	a b c	60 50 60			2 2	2		2		
7	a b c	50 58 58				2				
21	a b c	52 54 60	4 8		2	4				2
35	a b c	12 18 18	30 28 40		4 4					
49	a b c		84 94 92							2 2
63	a b c	2	22 16 38		2				100 96 94	4
77	a b c		8 2						100 100 100	30 20
84	a b c		8 2 <u>18</u>	22					92 94 100	4

 Table D.1.
 Microflora reading on filter paper for test 1-1.

	· · · · · · · · · · · · · · · · · · ·			Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b	90 80				2 2				
	С	76				6				
4	а	52				6				
	b	54			2	2				
	С	50						2		
7	а	40				4				
	b	42				4				
	С	40				4	2			
21	а	68				2				
	b	56	4			12				
	С	70	8							
35	а	64	2							
	b	42	2			6				
	С	44	4							
49	а	42	4							10
	b	32	16							4
	С	46	8			2				
63	а	26	10						42	
	b	12	2						80	
	С	22	10						68	
77	а	6		2					90	
	b								96	
	С	2							90	2
84	а	6	2						68	6
	b	8							82	
	С	2							96	

Table D.2. Microflora reading on filter paper for test 1-2.

			Microflo	ora % re	adings				
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	70 64 78			6				
4	a b c	56 46 68		2 2	2 2 2				
7	a b c	48 30 60			2				
21	a b c	56 62 54		2	2 4				
35	a b c	68 68 56	2	4	4 4				
49	a b c	82 60 80	2	2 6	4 2				
63	a b c	52 68 50	24 2	4	2				
77	a b c	6 58 20	16 2 10	2					2
84	a b c	48 28 54	6 2 30					6 30 48	16 14 18

 Table D.3.
 Microflora reading on filter paper for test 1-3.

.

			Microfic	ora % re	adings				
Day	Replicate	Alt	A.gl A.ochr	Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	82 62 58		2	2 2	2 2			
4	a b c	62 42 58		2 2	10 8				
7	a b c	52 66 54			2 2				
21	a b c	70 84 72			4 4 2	2 2	2		
35	a b c	70 66 82	2		2 4 2	2 2			
49	a b c	58 66 56		4	4 6 4				6 8
63	a b c	80 66 64	8 2	2	6	2			10 16
77	a b c	26 34 64	10 2		16 6	2			
84	a b c	68 54 60	22 2		10 6			16 6 8	12 4

		Microflora % readings							
_Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	84 70 54		2 4	2 4 2	2			
4	a b c	42 50 46		2	10 6 4				
7	a b c	60 52 52			8 4				
21	a b c	88 78 84		2	2	2	2		
35	a b c	62 84 72			2 2	_	4 6		2 4
49	a b C	74 72 88		2 4	4 2 2	2			6
63	a b c	44 76 30			10 10				
77	a b c	48 58 62			10 6 2	2 2 2			2 4 2
84	a b c	60 70 48	2 4		4 6 18			2	24 42 18

	Table	D.5.	Microflora	reading of	on filter	paper	for test 1-5.			
--	-------	------	------------	------------	-----------	-------	---------------			
		Microflora % readings								
-----	-------------	-----------------------	----------	---------	-------------	-------------	-------------	-----	----------------	----------------
Day	Replicate	Alt	A.gl	A.ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	64 72 38			8 6 6	2 8 4	2 2 2			
4	a b c	72 46 48				2	4			
7	a b c	38 48 42			2	2 2				
21	a b c	72 66 66				2 6 6	2			
35	a b c	64 70 38				2 2		2		
49	a b c	64 40 52			2 4 2	2	2			4 18 16
63	a b c	48 34 26	6 32		16 2	6				
77	a b c	30 22 14	10 20	2 16					74 8 2	
84	a b c	48 30 16	2 2			4			58 60 56	22 20 40

Table D.6. Microflora reading on filter paper for test 1-6.

and the second se			Microfle	ora % re	eadings	;			
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	54 68 86		2 4	4 6				
4	a b c	40 50 62		2	2 2	2			
7	a b c	48 50 50			4 2				·
21	a b c	84 72 64	4	6	6 4 6	2			6
35	a b c	72 66 60	4 6		2	2			
49	a b c	66 62 46	4 8	4 2	4				6 4
63	a b c	44 48 36	6 6 6	4	6 6 4				
77	a b c	48 38 26	2 2 6		8 12	2	10		4 6
84	a b c	66 70 44	8 26 70	2		2 2		14	18 10 10

Table D.7. Microflora reading on filter paper for test 1-7.

.			Microflo	ra % re	adings				
Day	Replicate	Alt	A.gl A.ochr	Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	а	82		6	4				
	b	84							
	С	64							
4	а	32		24					
	b	46			2	2			
	С	46	·		2	2			
7	а	28		32					
	b	52							
	С	66		2	2				
21	а	70		4	12				
	b	72		2	2				
	С	78		2					
35	а	56							
	b	74		2	2				
	С	92		2	2				
49	а	42	4		2				6
	b	56	2	2	8				
	С	52			2				10
63	а	44	16		8				2
	b	66	6			6		4	
	С	50							
77	а	26	4						2
	b	46	6						8
	С	14							10
84	а	4	18						30
	b		48					20	64
	С		38					24	38

 Table D.8.
 Microflora reading on filter paper for test 1-8.

			Microflo	ora % re	adings	-		· · · · · ·	
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	52 68 32		4 6 6	6 2 2				
4	a b c	52 66 58		2 2	2 4				
7	a b c	38 42 58			4 8				
21	a b c	86 70 56	4	2 4	2 4				
35	a b c	74 66 74	4	4	8 6 4	4	2		
49	a b c	78 66 78	2 22		4 2 2	2		2	4
63	a b c	60 56 52	2 18		2 10 4				10
77	a b c	24 56 16			6 10				
84	a b c	78 68 62			6 6 2				10

Table D.9. Microflora reading on filter paper for test 1-9.

			Microfl	ora % re	adings	;			
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	70 52 72		4 6	6 4 6		2		
4	a b c	50 48 54		12 2	8 4	2 2			
7	a b c	56 38 62			4 2				
21	a b c	80 54 74			4 8	2			
35	a b c	78 88 56	6		2 6				4 10
49	a b c	34 54 44	14		6 2				2
63	a b c	54 62 66	2 2 4	2	8 8 6				8
77	a b c	40 50 40	46 36		8 8				
84	a b c								d.c. d.c. d.c.

 Table D.10.
 Microflora reading on filter paper for test 1-10.

d.c. Indicates petri dish covered with this microflora thereby disabling any readings of other microflora.

			Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	70 66 60		4 2	6 6				
4	a b c	48 62 48			2 6				
7	a b c	64 62 52	• •	4	2 2 6				
21	a b c	44 76 76	14. 1		8 4				
35	a b c	82 76 72	4		2 2 4				
49	a b c	68 62 52		4	2 8 8	2			6
63	a b c	68 62 60	10 2	2	8 4				
77	a b c	44 24 60	6		4 4 10	2			22
84	a b c	44 32 70			2 4 4				14

 Table D.11. Microflora reading on filter paper for test 1-11.

		No. 100	Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	48 76 56			4 2 2	,			
4	a b c	52 28 46		2	2 2				
7	a b c	66 56 64			2 12	4			
21	a b c	70 74 50		2	2 2 14				
35	a b c	64 76 72			4	2			20 8
49	a b c	46 50 44	4	2 2	2 12 14				
63	a b c	52 64 52	4	4 2 2	6 8	2 2			10 12
77	a b c	42 24 18	2		4 2				
84	a b c	58 56 46			8 2				2 4

 Table D.12.
 Microflora reading on filter paper for test 1-12.

				Microflo	ora % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H:Sat	Muc	Pen	Rhiz
0	a b c	68 62 70				6 2 2				8 34 8
14	a b c	72 54 42	72 12 38		6		2		2	6 14 4
21	a b c	30 16 36	36 16 58						50 24 20	
35	a b c	6 12 6	40 44 18						100 98 92	
49	a b c	12 0 0	8 24 2			12 4			96 90 100	
63	a b c	2 0 0	58 64 18					2	64 80 100	100 6 2
77	a b c	2 n.d. 0	22 n.d. 8						98 n.d. 90	
91	a b c								100 n.d. 100	

Table D.13. Microflora reading on filter paper:	' for	or test 2	-1.
-------------------------------------------------	-------	-----------	-----

n.d. Indicates no data.

				Microflo	ra % re	adings	;		
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc Pen	Rhiz
0	а	76				6			2
	b	64				6			4
	С	62				10			8
14	а	58	14			8			2
	b	44	34				2	2	
	С	56	4						2
21	а	54	50					34	
	b	34	40					24	
	С	38	62					58	
35	а	30	18					86	
	b	20	12					88	14
	С	12	12					92	
49	а	22	2					90	
	b	10	6					92	
	С	8	0					90	2
63	а	10	8					82	
	b	20	14					94	
	С	10	4					94	
77	а	10						82	
	b	8						50	
	C	0	6					94	
91	a	40						84	
	b	24	-					96	
	С	10	_2					94	14

TANG D.14. WILLUNULA LEAUNU ON THE DADER IOF LEST A	Table D.14.	Microflora reading	on filter	paper for test 2-	2
-----------------------------------------------------	-------------	--------------------	-----------	-------------------	---

				Microflo	ra % re	adings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	64 58 52				10 4 14				6
14	a b c	48 50 58	76 20 12			2	2		10 2 2	18
21	a b c	40 20 26	84 74 58						2 24 10	
35	a b c	18 24	50 12 6		2		2 2		46 96 90	16 8
49	a b c	6 10 8	34 4						100 96 96	10
63	a b c	4 12 6	20 6 2					20 14	98 88 98	10 50
77	a b c	2 6							86 90 90	4
91	a b c	2 10 10	44 2 2						96 96 100	

	Table D.15.	Microflora	reading	on filter	paper for t	est 2-3.
--	-------------	------------	---------	-----------	-------------	----------

			Microff	0.00 0/ 00	o din a a				
Dav	Renlicate	Λ <i>Η</i>		<u>500</u>	Eug	LI Sot	Mula	Den	D6:-
Day	Teplicate	71	A.YI A.UUIII	Epc	rus	п.Sal	WIUC.	Pen	RIIIZ
0	а	40			2				
	b	38			12				2
	С	18							64
14	а	64	66						
	b	70	18		4				
	С	66	24						
21	а	84	14					62	
	b	54	58					44	
	С	70	26					2	
35	а	20	14					82	
	b	12	8			6		88	
	С	16	10	2				86	
49	а	16	0					94	
	b	10	8					94	2
	С	10	0					94	
63	а	34	6			2		86	
	b	20	12				2	88	50
	С	16	4					92	100
77	а	48						88	
	b	32						96	
	С	36						100	
91	а	16						80	
	b	8						78	14
	с	0						100	12

Table D.16. Microflora reading on filter paper for test 2-4.

			N	/licroflo	ra % re	adings				
Day	Replicate	Alt	A.gl A	A.ochr	Epc	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	46 64 48				2 4				18
14	a b c	10 64 50	62 8 30			2			22	28 4
21	a b c	74 58 76	22 8 16				2		10 0 18	
35	a b c	46 48 16	10 16 4						74 66 68	
49	a b c	22 20 22	2			4	2		86 78 82	4
63	a b c	40 36 26	24 2				4		86 86 94	50 50
77	a b c	20 24 44	8						96 76 96	
91	a b c	20 32 16	10 12 4						84 92 78	

Table D.17. Microflora reading on filter paper for test 2-5.

				Microflo	ra % re	adings				- 110
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	68 42 36				10 6	4		24 2	
14	a b c	76 58 64	4 12		4	2				6
21	a b c	34 66 38	52 6 40							10 4
35	a b c	28 26 22	20 24 10						4 40 38	16
49	a b c	36 48 22	16					2	48 70 92	4
63	a b c	54 46 34	16 2						76 76 94	6
77	a b c	40 20 60	2						64 72 88	6
91	a b c	16 38 24	14 8 10				2		12 38 84	2

Table D.18. Microflora reading on filter paper for test 2-6.

			Microf	lora % r	eadings	;			
Day	Replicate	Alt	A.gl A.och	r Epc	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	54 48 50	2	2	6 10 4				2 2 4
14	a b c	70 58 68	8 32 26	2	2	2		6	
21	a b c	36 28 58	16 14 26		6 4			6 8 4	22
35	a b c	20 28 26	56 52 42					4 4 4	
49	a b c	26 28 24	48	2			4	92 86 48	10 2
63	a b c	32 54 46	2 2					88 90 72	6 14
77	a b c	24 48 36	4 24					84 92 76	8
91	a · b c	8 14 16	4 4 28			2		74 50 56	12

Table D.19	9. Microflora	reading o	on filter	paper for	test 2-7

				Microflo	ra % re	adings			· · · · · · · · ·	
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	a b c	52 64 68			2	4 10				18
14	a b c	62 66 60	18 12			2			4	
21	a b c	34 60 38	6 30 18				2		16 32	
35	a b c	20 26 10	38 26 26				2		12 28 68	
49	a b c	12 6 14							70 80 90	
63	a b c	36 32 20	0 0 0						90 96 92	2
77	a b c	30 10 32							94 80 96	6 4
91	a b c	0 42 22	2 2 8						54 74 66	

Table D.20. Microflora reading on filter paper for test 2-8.

			Microflo	ora % re	adings				
_ Day	Replicate	Alt	A.gl A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	a b c	18 4		14					68 92 90
14	a b c	38 58 54	8 6 38		6				
21	a b c	60 52 68	26 8 16		2			20 4 14	
35	a b c	8 20 16	18 16 48					78 28 28	
49	a b c	22 26 28	82 92 74					82 92 74	4
63	a b c	56 64 48						98 88 88	6
77	a b c	54 50 54	12					86 88 92	14 14 18
91	a b c	32 34 36	12 6 6					74 72 62	

Table D.21. Microflora reading on filter paper for test 2-9.

				Microflo	ra % re	adings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	а	32								40
	b	60			4					
	С	56			8					2
14	а	64	12							
	b	62	2		6	4				
	С	62	8		2					
21	а	50	38		6				2	14
	b	66	22							
	С	64	54							22
35	а	50	46						22	
	b	34	60						10	
	С	40	36						16	6
49	а	36	72						8	
	b	40	70						10	
	С	38	76		4			4	4	
63	а	52							90	
	b	50							72	
	С	50	20						54	10
77	а	62	4						44	70
	b	64	2			2			64	56
	С	46	8						58	18
91	а	28	26						28	
	b	24	36						26	8
	С	54	24						32	10

Table D.22. Microflora reading on filter paper for test 2-10.

				Microflo	ora % re	adings	;			
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Мис	Pen	Rhiz
0	а	42				6				
	b	60				6				2
	С	46				4				
14	а									100
	b	24	8			4				100
	С	68								100
21	а	50	12							
	b	52	46			4				
	С	58	24			4	2			
35	а	36	38						16	
	b	46	24						6	100
	С	20	30							100
49	a	22	54							
	b	30	64						2	
	С	50	52						2	
63	а	40	16						8	
	b	42	4						28	
	С	50	10						38	
77	а	34							88	52
	b	32						16	66	70
	С	62	4			2		4	52	12
91	а	54	24					16	18	50
	b	54	32				2	64	30	4
	С	46				2	4	4	62	

Table D.23. Microfiora reading on filter paper to	or test 2	-11.
---------------------------------------------------	-----------	------

ŝ

				Microflo	ra % re	eadings				
Day	Replicate	Alt	A.gl	A.ochr	Ерс	Fus	H.Sat	Muc	Pen	Rhiz
0	а	40								44
	b	58			2	4				2
	С	56				8				
14	а	56	26			2				2
	b	66	44			2	2			6
	С	22	14							2
21	а	50	16						30	
	b	26	40							
	С	48	44			16	2			
35	а	50	20						8	34
	b	26	38						4	20
	С	26	42							
49	а	32	80						18	
	b	12	38							
	С	12	42						2	
63	а	38	2				2		80	8
	b	8	8					6	20	
	С	28	4					4	24	2
77	а	32							66	10
	b	22						16	84	18
	С	38	2					34	68	
91	а	42	8						78	
	b	32	10						82	
	С	30	2						58	

Table D.24. Microflora reading on filter paper for test 2-12.

APPENDIX E: Fat acidity value data.

		Storage day								
Test	Replicate	0	4	7	21	35	49	63	77	84
1	a	9.52	6.71	7.19	13.42	12.70	22.04	45.04	57.50	55.58
	b	9.82	6.47	8.15	12.69	10.78	23.00	41.45	57.02	57.02
	c	8.86	6.47	8.86	11.50	11.98	21.56	47.92	48.04	58.46
2	a	7.67	6.71	6.95	10.30	10.78	15.81	26.11	36.66	41.21
	b	7.19	6.71	6.71	11.74	11.26	14.61	31.15	41.21	42.88
	c	7.91	7.67	9.58	10.78	11.74	13.18	31.15	39.77	40.25
3	a	6.47	5.75	8.15	9.10	9.10	10.78	17.73	23.72	30.19
	b	6.23	6.47	6.47	9.58	10.06	9.34	15.09	15.81	20.36
	c	6.71	6.47	6.95	9.82	10.54	10.30	18.21	25.16	17.49
4	a	7.91	5.75	7.43	9.58	8.86	9.82	10.54	12.22	17.73
	b	6.95	6.71	7.43	7.91	7.19	9.10	12.46	20.60	13.42
	c	8.62	5.75	7.43	8.86	7.67	8.86	11.02	11.26	26.83
5	a	6.47	7.19	7.91	10.30	5.99	7.19	8.50	9.58	9.82
	b	5.99	7.19	6.95	7.67	6.23	7.19	9.58	10.06	9.34
	c	6.95	7.43	8.86	7.19	6.95	6.95	7.43	9.58	8.86
6	a	6.71	8.15	9.82	9.58	9.58	9.82	15.57	26.11	34.50
	b	6.23	7.43	7.91	9.10	10.06	10.30	17.73	28.27	32.34
	c	7.67	7.91	5.99	9.82	10.06	9.82	24.44	33.30	33.50
7	a	8.39	6.23	7.91	9.10	9.82	6.47	8.62	10.54	12.70
	b	7.19	7.43	7.19	8.39	8.86	8.62	8.86	14.85	13.66
	c	8.15	7.91	9.82	8.15	8.62	9.58	9.82	22.28	25.16
8	a	6.23	7.91	8.39	9.10	6.47	11.02	14.85	20.84	22.28
	b	6.23	7.19	9.34	8.86	7.43	6.95	12.70	23.96	23.00
	c	6.47	8.15	8.62	8.39	7.91	9.34	11.02	17.73	21.32
9	a	6.47	8.86	8.15	7.19	8.15	9.34	8.39	10.06	8.62
	b	6.71	6.95	8.86	9.34	9.10	7.67	8.39	9.34	11.26
	c	6.23	7.43	10.30	11.02	9.34	8.39	10.30	9.82	9.82
10	a	5.75	7.67	8.62	6.71	8.86	8.39	9.58	15.09	18.45
	b	6.23	7.43	10.30	6.47	9.10	9.34	9.34	14.85	18.69
	c	6.23	6.95	9.82	6.23	9.34	9.58	10.54	23.48	31.62
11	a	5.75	7.19	8.15	7.43	8.86	9.58	11.50	16.77	20.12
	b	6.23	7.19	9.58	7.67	9.58	9.58	11.02	13.18	18.45
	c	6.71	7.43	9.58	9.82	11.02	8.62	12.70	13.90	13.90
12	a	7.19	6.47	7.67	8.15	11.02	9.58	8.62	9.10	12.46
	b	5.99	6.71	7.19	7.67	10.06	9.58	11.74	9.58	12.46
	c	6.23	6.47	7.91	8.62	10.54	8.62	8.15	11.98	11.02

Table E.1. Fat acidity value of grain in set 1.

		Storage day							
Test	Replicate	0	14	21	35	49	63	91	
1	a	8.38	16.77	35.22	54.86	75.95	68.28	69.96	
	b	7.91	11.74	38.57	63.97	74.75	60.13	n.d.	
	c	10.30	16.53	29.23	57.26	75.71	67.08	64.21	
2	a	10.78	8.86	20.12	40.25	56.30	51.27	62.77	
	b	10.54	11.26	25.16	41.45	51.99	56.06	54.38	
	c	8.38	11.50	30.43	50.79	56.78	57.50	62.77	
3	a	9.10	12.46	35.94	43.84	45.52	45.28	64.93	
	b	10.30	10.06	30.19	44.32	45.52	50.07	71.16	
	c	9.58	16.77	31.86	46.72	47.92	50.31	59.18	
4	a	8.38	18.93	35.94	43.36	51.27	48.63	52.95	
	b	8.86	12.70	32.34	44.32	55.34	58.46	n.d.	
	c	10.54	12.22	n.d.	44.56	54.62	55.82	64.93	
5	a	8.38	8.15	16.29	40.49	51.75	51.27	64.21	
	b	8.86	11.26	12.46	35.46	55.43	53.19	68.76	
	c	8.38	10.06	13.66	33.78	47.92	52.47	56.54	
6	a	9.10	10.06	11.98	28.51	33.06	31.15	36.66	
	b	9.10	10.06	13.90	27.31	30.67	28.75	n.d.	
	c	7.67	8.86	17.01	32.58	32.58	34.74	45.52	
7	a	7.43	11.26	12.46	32.10	36.18	40.73	49.11	
	b	7.91	12.70	22.28	34.26	35.94	35.94	48.87	
	c	8.15	9.58	21.32	n.d.	31.38	35.94	48.16	
8	a	9.10	9.58	17.49	30.43	32.10	39.77	49.35	
	b	8.38	8.62	16.77	35.70	41.45	48.16	54.38	
	c	8.86	9.58	22.52	38.57	51.75	44.56	60.13	
9	a	9.34	9.58	19.65	38.09	46.00	43.36	53.67	
	b	8.62	11.26	19.17	32.58	38.57	38.09	45.04	
	c	7.43	10.54	17.49	33.30	32.82	39.05	48.16	
10	a	8.38	8.62	13.18	22.76	30.19	34.02	35.46	
	b	9.34	8.62	10.54	25.87	31.38	27.31	35.94	
	c	7.91	8.39	12.22	18.45	26.59	30.67	35.46	
11	a	8.15	9.34	9.34	23.48	29.95	36.18	45.28	
	b	9.10	6.71	11.98	28.03	30.43	34.98	40.01	
	c	9.10	8.62	11.74	22.04	28.75	32.82	38.33	
12	a	6.95	n.d.	n.d.	34.98	41.93	43.12	49.83	
	b	9.10	11.02	17.73	27.55	42.41	40.73	51.99	
	c	9.58	11.26	14.14	n.d.	36.66	39.53	49.11	

 Table E.2.
 Fat acidity value of grain in set 2.

n.d. Indicates no data.

120

APPENDIX F: Moisture content data.

	Storage day								
Test	0	7	21	35	42	49	63	77	84
1	16.8	16.6	14.7	13.8	15.1	16.5	17.1	16.1	14.8
2	17.1	16.7	15.4	14.8	16.3	16.8	16.9	17.2	18.0
3	16.9	16.6	16.0	16.3	16.5	16.7	16.3	16.7	17.2
4	16.8	17.0	16.5	16.6	16.9	17.1	17.6	17.3	18.0
5	16.7	16.8	16.8	16.8	17.1	17.5	17.5	17.5	17.6
6	16.9	16.5	15.5	15.6	16.2	16.7	16.4	17.0	17.2
7	16.9	16.5	16.2	16.3	16.4	16.9	16.9	17.1	17.3
8	16.8	16.6	16.1	16.2	16.7	17.2	17.5	17.6	18.9
9	16.9	16.6	16.1	16.2	16.5	16.8	17.1	17.1	17.1
10	17.0	16.5	16.3	16.1	16.4	17.1	17.3	17.6	18.6
11	16.9	16.8	16.0	16.4	16.8	17.1	17.2	17.3	17.6
12	16.7	16.5	16.1	16.2	16.4	17.0	17.5	17.5	17.6

 Table F.1. Moisture content of each test in set 1 throughout the experiment.

Table F.2. Moisture content of each test in set 2 throughout the experiment.

	Storage day							
Test	0	14	21	35	49	63	77	91
1	16.5	16.2	17.0	16.6	17.4	16.5	14.9	16.5
2	16.6	15.9	17.4	16.9	17.2	17.3	16.1	16.6
3	16.7	16.1	17.2	16.6	17.6	17.8	17.0	16.4
4	16.3	16.4	16.6	16.8	17.5	17.6	17.2	17.0
5	17.0	16.2	17.5	17.3	18.9	17.7	17.4	16.7
6	16.4	15.4	17.2	18.0	18.1	18.0	17.1	16.7
7	16.5	16.4	16.1	17.7	17.9	17.2	16.1	17.2
8	16.4	16.3	17.0	17.5	16.7	16.2	15.7	17.0
9	16.5	15.8	17.2	17.5	17.2	17.2	16.8	16.8
10	16.3	16.6	17.6	18.1	18.1	18.0	17.6	17.1
11	16.5	16.2	18.5	18.8	18.9	18.1	17.4	16.6
12	16.0	14.6	16.5	18.3	19.0	18.4	18.3	17.5