A systemic approach of qualitative changes in the stored wheat ecosystem: prediction of deterioration risks in unsafe storage conditions in relation to relative humidity, infestation by *Sitophilus oryzae* (L.), and variety influence

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Abstract

A multidimensional laboratory trial was carried out to identify how key overall quality traits of different common wheat varieties change during storage, to understand their interactions in the process of deterioration, and finally to reveal underlying trends of critical storage conditions that may endanger grain quality. A large set of qualitative criteria were followed on grain batches of three wheat varieties with various qualities for food processing, which were stored for 160 d at 22-23°C, under two different relative humidities (r.h.), and with or without infestation by the rice weevil *Sitophilus oryzae*. All variables involved in quality components assessment were recorded at 42-d periods. From the Pearson's product moment correlation matrix, it was observed that the quality traits that correlated significantly to biotic variables (insect and fungal species dynamics) were: moisture content, hL mass, seed viability, and fat acidity of extracted flour. The interactions between biotic deteriorative factors and qualitative trait changes revealed through principal component analysis (PCA) were significant between four factors explaining the major part of qualitative criteria variance: storage duration; moisture content; hidden infestation density; fungal contamination level. The rate of increase of insect population was significantly different among varieties. PCA revealed that the significant difference in qualitative deterioration pattern among the three varieties was not related to their hardness, but to a different r.h. affinity. The germination rate was the qualitative criteria the more early declining during storage. The technological properties of extracted flour from each variety were significantly affected only when insect density exceeded 1000 insects per kg, a situation only observed in hot-spots. This work highlighted the trends of variation in quality traits of wheat varieties when stored under critical conditions. It can be used in IPM approaches to predict the susceptibility of a wheat variety to insect and fungi damage during storage.

Keywords: Common wheat, Variety, Qualitative change, Insect pest, Fungal spoilage, Multivariate analysis

1. Introduction

The interactions between quality biodeterioration factors (insects and fungi) and the quality traits that determine the commercial value of a grain batch are complex. Variable qualitative damage may occur according to storage conditions and the intrinsic sensitivity of the species and variety of cereal to these pests, as we have shown in previous work (Bekon and Fleurat-Lessard, 1992; Fourar, 1994; Fourar and Fleurat-Lessard, 1997). The conditions of safe storage may dramatically change when grain batches kept in good and safe storage conditions in wheat-producing countries are exported to other countries with much warmer climates and sometimes no availability of the adequate means to manage storage pests. Thus, to develop a better understanding of the susceptibility of different wheat varieties or cultivars to qualitative deterioration during storage, the objectives of this research were: i/comparison of the storability of different wheat varieties currently cultivated in France; and ii/identification of the major deterioration factors of the overall quality traits in order to understand the role of their interactions in the deterioration rate, especially for the more sensitive wheat quality traits.

2. Material and methods

A multidimensional laboratory trial was carried out to identify what key factors of the overall quality traits change, to understand their interactions in the process of deterioration, and finally to reveal underlying trends of critical storage conditions that may endanger grain quality retention. A large set of qualitative criteria were followed on grain batches from three wheat varieties with various qualities for cereal food processing. Wheat was stored for 160 d at 22-23°C, under two different relative humidities (r.h.), and with or without infestation by the rice weevil *Sitophilus oryzae* (L.). Variables involved in quality determination were recorded approximately every 40 d to build the multivariate data matrix (Tab. 1).

Table 1 List of quality traits of wheat grain and reference of each analytical method used for their quantification in the present study.

Grain Quality trait analyse	acronym	Analytical method	Reference
1. Sanitary and soundness condition			
Adult insects counting	Insect_AD	Sieving – NF-V 03-742	AFNOR, 1982
Insect hidden infestation counting	Insect_HI	Radiograph-ISO 6632-4	AFNOR, 1982
2. Microbiological spoilage			
2.1 Qualitative analysis: Rate of fungi-contaminated kernel	Cont_Rate	Ulster's method	Cahagnier and Richard-Molard, 1997
2.2 Quantitative analysis: Isolation and identification of fungal colony-forming-unit (CFU) per g	Fungi_Q	NF V08-011	AFNOR, 1996a
3. Germination			
Germinative capacity	Germ_Cap	ISTA rules for seed testing	ISTA, 1999
4. Physical-chemical condition			
Moisture content (wet basis)	MC	Oven-drying practical method NF V 03-707	AFNOR, 1982
Kernel hardness	Hardness	Hardness point-meter	Hardness meter notice
Hectolitre mass (or volumic mass)	hL_M	NF V 03-719	AFNOR, 1996b
5. Biochemical composition			
Nitrogen and protein content	Prot	Dumas method PR NF EN ISO 16634	
Lipid acidity (or fat acidity)	Lipid_Ac	NF V 03-712	AFNOR, 1982
6. Technological criteria (or fitness)			
6.1 Laboratory mill processed products			
Hagberg index (falling number)	Hagb_Ind	NF V 03-703	AFNOR, 1982
6.2 Alveographic parameters			
Baking strength (W)	W	ISO method 27971	ISO, 2008b
Other parameters (P, G, P/L)	P, G, P/L	ISO method 27971	ISO, 2008b
7. Statistical analyses			
Multivariate explanatory analyses		Multiple correlation - PCA	Xlstat®

In this experimental design, we applied the "fixed-effect-modelling" approach in which several qualitatively and logically distinct variables were measured on the same grain samples. This is a covariance analysis situation where several treatments (different grain varieties and storage conditions) were applied to the objects of the experiment (grain samples) to see if the response variable values changed. This is also a situation in which several variables are measured on each individual. The statistical analysis of the quantitative data used the explanatory procedure of principal component analysis (PCA). The multidimensional statistical analysis and chart plotting were achieved with Xlstat® (Addinsoft, Paris, France) software.

In our experiment, multivariate data were expressed in a matrix form with p columns (measured variables) and n rows (individual grain sample partitioned into different classes (or "dependent variables"): variety, r.h. level, insect presence or absence). The data processing software allowed the calculation of simple and multiple correlation coefficients, and the analysis of covariance to model by multiple correlation the evolution of each dependant variable as a function of the explanatory variables. The Pearson' s product moment correlation matrix of the correlations between all the dependant and explanatory variables was built to appreciate their complex interactions, which were then graphically represented using two major components of PCA.

3. Results and discussion

3.1. Change in quality traits with storage time

From the Pearson's product moment correlation matrix, it was observed that the variables that correlated significantly to biotic variables (insect and fungal species dynamics) were: moisture content, 1000-grain mass, seed viability, fat acidity and Hagberg index of extracted whole flour. The interactions between biotic deteriorative factors and qualitative trait changes revealed through principal component analysis (PCA) were significant with four factors explaining the major part of the variance of qualitative criteria: storage time, moisture content, hidden insect infestation density, and fungal contamination level (Tab. 2). The rate of increase of insect population was significantly different among varieties (Tab. 3 and 4). PCA revealed that the significant difference in qualitative deterioration pattern observed among the three varieties was not related to their hardness, but rather to a different r.h. affinity. The qualitative traits which were more early affected during the storage period were germination rate and energy.

Table 2 Pairwise correlations between all variables relating quality attributes and "deterioration factors" of three wheat varieties stored during 160 d at 22-23°C, under two different r.h. conditions, and with and without an infestation with *Sitophilus oryzae* (Pearson' s product moment correlation matrix).

Variables	Hard-ness	PROT	RH_ Equi	Infested	Time	hL Mass	MC	Insect AD	Insect HI	Fungi Qu	Cont Rate	Fat Acid	Hagb Ind
Hardness	1												
PROT	-1.000	1											
RH_Equi	0.000	0.000	1										
Infested	0.000	0.000	0.000	1									
Time	0.000	0.000	0.000	0.000	1								
hL Mass	-0.158	0.158	-0.267	-0.246	-0.541	1							
MC	-0.086	0.087	0.286	0.256	0.671	-0.850	1						
Insect AD	-0.055	0.056	0.087	0.431	0.260	-0.249	0.364	1					
Insect HI	0.005	-0.005	0.073	0.330	0.389	-0.866	0.764	0.073	1				
Fungi Qu	0.203	-0.203	0.144	0.172	0.195	-0.790	0.613	-0.028	0.773	1			
Cont Rate	0.367	-0.368	0.195	0.046	-0.615	0.108	-0.311	-0.193	-0.194	0.214	1		
Fat Acid	0.033	-0.032	0.164	0.240	0.438	-0.911	0.818	0.145	0.917	0.909	-0.091	1	
Hagb Ind	0.676	-0.675	-0.007	0.004	-0.116	-0.073	0.038	-0.084	0.097	0.156	0.149	0.086	1

Values in bold are different from 0 at signification level alpha = 0.05

Table 3 Equations of exponential models predicting the rate of increase of the density of *Sitophilus oryzae* adults during 124 d development of two couples on the three wheat varieties at two r.h. levels: comparison of parameters derived from the calculated rate of increase in the different experimental conditions.

Variety	Equilibrium r.h. (%)	Exponential rate of increase equation	Net multiplication rate after 124 d	Natural rate of increase per d (rm)	Correlation coefficient R²
Caphorn	75	y = 0.518285 e0.054221x	144	0.0401	0.87
	85	y = 0.2252 e0.0709x	494	0.0500	0.94
Apache	75	y = 0.1538 e0.0778x	794	0.0538	0.97
	85	y = 0.2769 e0.0747x	973	0.0555	0.98
Crousty	75	y = 0.2607 e0.0684x	419	0.0487	0.95
	85	y = 0.1491 e0.078x	789	0.0538	0.98

Table 4 Equations of exponential models predicting the rate of increase of the density of *Sitophilus oryzae* hidden infestation (all stages) during 124 d development of two couples on three wheat varieties at two r.h. levels: comparison of parameters derived from the calculated rate of increase under the different experimental conditions.

Variety	Equilibrium r.h. (%)	Exponential rate of increase equation	Net multiplication rate after 124 d	Natural rate of increase per d (rm)	Correlation coefficient R²
Caphorn	75	y = 15.314 e0.0275x	463	0.0495	0.68
	85	y = 9.1155 e0.0361x	801	0.0539	0.91
Apache	75	y = 7.0759 e0.043x	1464	0.0588	0.96
	85	y = 10.398 e0.0427x	2072	0.0616	0.93
Crousty	75	y = 21.906 e0.0284x	741	0.0533	0.81
	85	y = 57.586 e0.023x	998	0.0557	0.92

From the alveograph® test results, measured only once at the end of the storage period, it was shown that storage of any of the three varieties at 75% r.h. did not significantly modify the flour baking strength (W), in spite of a small reduction of the swelling parameter (G) (Tab. 5). Flour extracted from infested samples compared to uninfested samples stored under the same r.h. conditions, had a clear reduction in W and G. In infested series, the extreme insect damage reached at the end of the storage period induced an imbalance of the r.h.eological parameters of the extracted flours. Thus, the technological properties of flour extracted from each variety were significantly affected only when insect population density exceeded 1000 adult insects per kg, a situation in practice only observed in hot-spots.

Table 5 Comparison of the alveographic parameters measured on flour extracted from the three wheat varieties before and after 160 d storage at constant temperature (22-23°C), at two r.h. levels and with and without an infestation by *Sitophilus. oryzae*.

Variety	Before storage			After 160 d of storage Conditions											
				75% r.h. no insects			75% r.h. with insects			85% r.h. no insects			85% r.h. with insects		
	W	G	P/L	W	G	P/L	W	G	P/L	W	G	P/L	W	G	P/L
Caphorn	240	18.1	1.39	270	17.7	1.68	195	12	5.07	280	18.1	1.55	185	12	4.97
Apache	255	24.5	0.51	235	23.8	0.52	105	15.1	1.41	165	19.4	0.72	NA	NA	NA
Crousty	190	27.4	0.25	120	20.6	0.44	120	19.7	0.55	135	23.3	0.32	155	14.8	1.84

3.2. Interactions between the studied variables

Although the hardness criterion is considered by many researchers as being related to varietal susceptibility to insect attack (Russel, 1962; Dobie, 1974; Juliano, 1981; Horber, 1983; Fourar, 1994), but even though the three varieties tested had large differences in hardness this was not the major factor involved in a lower level of susceptibility to insect attack and S. oryzae population multiplication. This contradiction could be clarified by multiple correlation modelling of each monitored qualitative criteria as a function of related explanatory variables. Thus, the variation in grain moisture content of the three wheat varieties can be modeled by four explanatory variables: r.h. equilibrium level, storage duration, insect infestation, and variety (Tab. 6). Storage time and variety influence were the most important variables explaining the major amount of variance explained by multiple linear regression. The variable `Hardness' did not show any dependence with the variation in insect density (neither adult nor hidden infestation). Thus, it could be shown that the hardness characteristic did not significantly influence the susceptibility of the three wheat varieties to the weevils, but that this susceptibility was rather related to capacity for r.h. absorption of grain.

Table 6 Multiple correlation modelling of qualitative traits (dependent variables) changes during storage time of three wheat varieties kept during 160 d in experimental storage at two different r.h. and with and without an infestation by *S. oryzae* (four quantitative explanatory variables and one qualitative variable: variety). The parameters of the linear (polynomial) models are indicated as "value" with the level of significance of the deviation from the mean (the constant term in the equation was omitted).

Explanatory variables	**# F-test value**	**Hardness**			**RH Equilibrium**			**Insect infestation**	
Dependent variables		**Value**	**t**	**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**	**Value**	**t**
Moisture content	21.263***	-0.012	-1.21	0.233	7.779	3.485	0.001***	0.695	3.115
Germination capacity	6.411***	0.02	0.123	0.9	-60.4	-1.614	0.114	-10.71	-2.86
Adult Insect density	3.181*	-2.888	-0.451	0.654	9905	0.663	0.511	490.86	3.283
Insect hidden infestation	3.843**	-0.886	-0.028	0.98	4258.5	0.573	0.569	1918	2.582
Fungi germs quantity	1.334 NS	41.69	1.392	0.171	7015	1.003	0.322	840.5	1.202
Seed contami-nation rate	12.262***	0.162	3.813	< 0.001***	19.583	1.978	0.055	0.458	0.463
Lipid acidity	3.577**	0	0.139	0.89	0.071	1.282	0.21	0.01	1.872
HL mass	6.9***	-0.041	-1.37	0.178	-16.33	-2.337	0.024*	-1.506	-2.155
Hagberg Index	24.86***	5.178	8.225	<0.001***	-12.292	-0.084	0.934	0.729	0.05

continue

Explanatory variables	**# F-test value**		**Storage time**			**Variety (global)**		
Dependent variables		**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**	**Value**	**t**	**Pr. > [t]**
Moisture content	21.263***	0.003**	0.02	8.172	<0.001***	0.965	4.073	<0.001***
Germination capacity	6.411***	0.007**	-0.171	-4.125	<0.001***	-8.192	-2.161	0.045*
Adult Insect density	3.181*	0.002**	3.276	1.98	0.05*	121.5	0.766	0.448
Insect hidden infestation	3.843**	0.0013**	25.025	3.044	0.004**	1353.8	1.717	0.093
Fungi germs quantity	1.334 NS	0.236	10.551	1.363	0.18	441.78	0.595	0.555
Seed contami-nation rate	12.262***	0.646	-0.069	-6.256	<0.001***	-2.13	-2.027	0.049*
Lipid acidity	3.577**	0.068	0	3.214	0.003**	0.09	1.539	0.131
HL mass	6.9***	0.037*	-0.037	-4.728	<0.001***	-0.266	-0.359	0.722
Hagberg Index	24.86***	0.961	-0.352	-2.165	0.036*	107.21	6.87	<0.001***

*** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$. – # F value of multiple regression model (5, 42 df)

One of the original results of this study was to show that the greater sensitivity of the Apache variety to the multiplication and the damage caused by *S. oryzae* was primarily associated with the intrinsic physical-chemical property of this variety to equilibrate aw and r.h. at a higher level of moisture content than the two other varieties, for the same r.h. in the grain samples storage enclosure. Figure 1, which represents the circle of the correlations between all the measured quantitative variables showed a first component (F1) that contained 40.7% of the variability. The proximity of the factors of qualitative deterioration with one of the ends of the axis F1, highlights a strong negative impact of four variables representing the "deterioratives forces" (Tipples, 1995) (variable: Time, MC, Insect_HI, Fungi_Q) on three major criteria of grain sanitary condition (Fat_Acid, Germ_Cap and hL_M). The three variables related to sanitary condition (MC, Insect_HI and Fungi_Q) were positively correlated to the storage period (Time), indicating that lengthening the storage period simultaneously increased the grain's hydration level, that in turn increased insect hidden stages density (in the case of infested series), and enrichment of storage fungi germs. Yet, a significant evolution of fungal contamination level was only observed during the last month of storage and was induced by the huge insect infestation level.

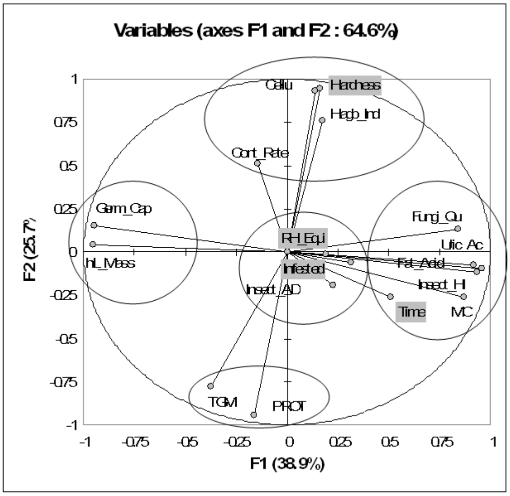

Figure 1 PCA: circular diagram of correlations between all variables (dependent and *explanatory* variables; these later within a shaded frame) revealing the interactions in qualitative trait changes occurring during a 160-d storage period (*Time*) of three wheat varieties of different hardness (*Hardness*), at two different r.h. levels (*RH_Equi*), and with or without an infestation by *Sitophilus oryzae* (*Infested*).

The second component (vertical axis) of the PCA, which included nearly 21% of the global variance, was directed by the physical-chemical variables related to intrinsic quality: hardness, protein content, and Hagberg index (Hi). The hidden infestation density was positively correlated with some dependent variables (MC, Lipid_Acid, Fungi_Q) and negatively with others (Germ_Cap and hL_M). The correlation with Time as the main explanatory variable related to insect hidden infestation variance was also highlighted through the PCA diagram.

3.3. Concluding remarks

In our study, the differences in susceptibility of wheat varieties to adverse storage conditions (high ambient r.h., high temperature and insect infestation) could be related to the dynamics of the interactions between ‘deteriorative forces’ and qualitative traits. The prediction of quality traits deterioration risks for different wheat varieties should be useful in building preventive strategies of grain storage management, especially in Mediterranean or tropical countries having imported wheat for long-term storage or strategic reserves.

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