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TOEGEPASTE BIOLOGISCHE WETENSCHAPPEN

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Concerning: final report of storage experiment for 6 months of cacao beans packaged in vacuum system

The cacao beans were vacuum packaged. To simulate extreme climate conditions, the packages were stored in closed boxes with 80% relative humidity and at 30°C.

The following quality parameters of the beans were determined in threefold for the initial quality, in twofold for two packages after one, two, three and six months of storage:

- gas concentration
- visual appearance (external and internal, internal disorders)
- water activity
- moisture content
- pH
- titritable acid (meq NaOH/100 g beans) (TA)
- peroxide value (meq O_2/ kg fat) (PO)
- free fatty acids (FFA, %)

1. Gas concentration

The results of the headspace gas concentration are tabulated in **Table 1**. The concentration of O_2 and CO_2 was measured by gas chromatography.

Table 1. Gas concentration (%) in vacuum packages of cacao beans, stored at 30°C and 80% RH

Storage period	Package 1		Package 2	
	% O_2	% CO_2	% O_2	% CO_2
Month 1	0.78	1.19	0.43	0.96
Month 2	0.04	2.94	0.00	2.96
Month 3	1.76	0.00	0.72	0.00
Month 6	0.96	0.36	0.42	0.00

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Initially, the packages were completely vacuum. During storage, small variations in gas concentration were measured between the different bags, but no accumulation of O_2 was observed. The O_2 permeability of the applied packaging film is very low, in order to prevent an increase in the O_2 concentration. CO_2 can be produced during storage of plant material due to their respiration activity. As the cacao beans were a dry product, no respiratory activity was expected. Indeed, no accumulation of CO_2 could be measured during the different months of storage in the headspace of the vacuum packages.

Conclusion: the barrier properties of the packaging film are strong enough to prevent an increase of O_2 inside the packages. No accumulation of CO_2 occurred because the beans were dry. The gas concentration of the headspace inside the vacuum packages stayed stable during the storage of 6 months in the extreme climate conditions (30°C and 80% RH). The vacuum conditions were not lost during the storage period due to the strong barrier properties of the applied packaging film.

2. Visual appearance (external and internal, internal disorders)

As visual appearance of the beans several aspects were followed:

- visual external mould development
- visual internal mould development
- visual external disorders
- visual internal disorders
- internal insects, eggs of insects,...
- colour of the beans (amount of violet beans)

In order to evaluate these properties, 20 beans from each sample were visually analysed. For each vacuum package, a sample was taken at the surface of the package and in the middle of the package. Consequenlty, it was possible to evaluate the gradient of quality degradation from outside to the kernel of the vacuum package. The results are expressed in % and are the average of 2 packages. The individual scores per package can be found in the different month reports. To evaluate the internal properties, the beans were split with a sharp knife. Results are summarised in **Table 2**.

Table 2. Visual appearance (%) of cacao beans, stored in vacuum packages, at 30°C and 80% RH

	Month 0	Month 1		Month 2		Month 3		Month 6	
		surface	middle	surface	middle	surface	middle	surface	middle
External mouldiness	0	2.5	0	7.5	5	0	0	0	0
External disorders	15	0	5	10	5	17.5	12.5	17.5	10
Internal mouldiness	1.67	5	5	7.5	0	15	10	2.5	2.5
Internal disorders	8.33	7.5	7.5	15	15	7.5	15	17.5	25
Internal insects	0	0	0	0	0	0	0	0	0
Violet beans	15	20	20	17.5	20	22.5	22.5	12.5	15

External mouldiness was found very limited and incidential. No systematic visual mould development was observed on the beans during storage in the vacuum packages and the extreme climate conditions.

Internal mouldiness was found as well very limited and incidential. No systematic visual mould development was observed in the beans during storage in the vacuum packages and the extreme climate conditions. No difference is found between the samples taken at the surface or in the middle of the vacuum package.

The percentage of **external disorders** did not change during the storage period. Big variations were detected between the different samples.

The percentage of **internal disorders** increased during storage. A trend of more internal disorders were detected at the end of the storage experiment in extreme climate conditions and also more were found in the sample, taken from the middle of the vacuum package. But this can be attributed to the big variability between the individual beans.

No **internal insects** or insect eggs were visually found during the storage period. If any insect or insect eggs were present between or in the beans, no growth or survival can be expected because of the low O_2 concentration inside the packages.

The amount of **violet beans** varied between the different months but no difference was found between the samples from the middle or from the surface of the vacuum package. The storage period and storage conditions did not influence the colour of the beans.

Conclusion:

No systematic trend (increase or decrease) in the different aspects of the visual quality of the cacao beans could be detected during the 6 months of storage in the Vacuum Packaging System, stored in extreme climate conditions (30°C and 80% RH).

3. Water activity

The water activity of the beans was measured as well in duplicate from one sample at the surface and one sample from the middle of the vacuum package. For every analysing point, two vacuum packages were analysed. In Figure 1 the evolution of the water activity is illustrated over the storage period.

Out of Figure 1 it can be concluded that the water activity is increasing during the storage period. But the critical value of 0.60 is not reached after 6 months of storage (a water activity of 0.60 is limit for development of moulds). Out of the sorption isotherm it was concluded that a relatively flat relation was found between the water activity and the moisture content in the region between 0.46 and 0.60 water activity.

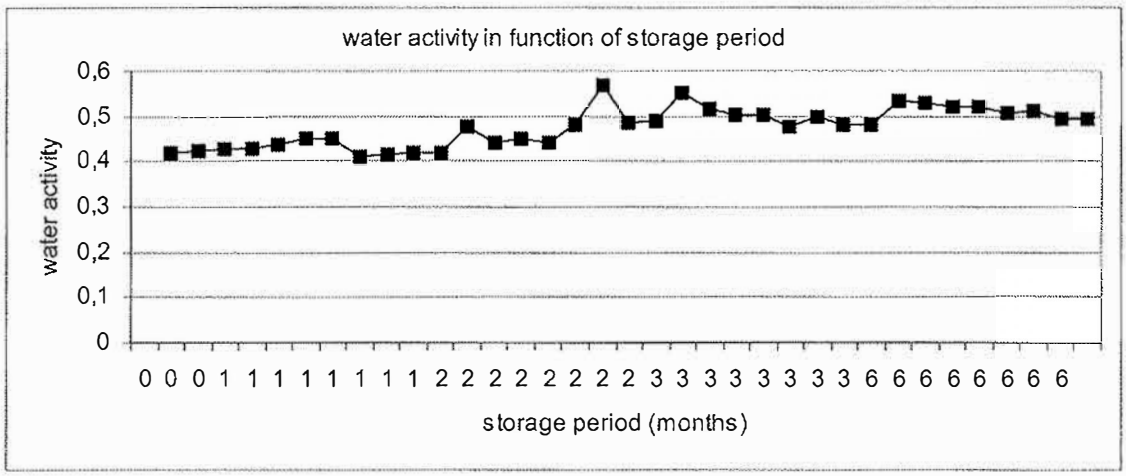

Figure 1. Water activity of the cacao beans in function of storage period, stored in a vacuum package under extreme climate conditions (30°C and 80% RH).

With the statistical program SPSS an independent T-test was done in order:

(1) to evaluate the difference in water activity between the different months (month 1, 2, 3 and 6 were compared with the initial quality (month 0)) (= influence of storage period)
(2) to evaluate the difference between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 3** the average are given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 3. Statistical interpretation of the evolution of the water activity of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average water activity	Statistical conclusion	Average water activity of sample at the surface of the package	Average water activity of sample at the middle of the package	Statistical conclusion
Month 0	0.42				
Month 1	0.43	No significant difference	0.42	0.43	No significant difference
Month 2	0.48	Significant difference	0.49	0.47	No significant difference.
Month 3	0.50	Significant difference	0.51	0.49	No significant difference
Month 6	0.51	Significant difference	0.52	0.51	No significant difference

Between month 0 and month 1 no significant difference was detected in water activity. But, during further storage, the water activity increased significantly.

The water activity of the sample at the surface was slightly higher compared to the water activity of the sample in the middle of the vacuum package, but this difference was not significant.

Conclusion: The barrier properties of the packaging film for water vapour is very high, in these extreme climate conditions, water vapour could transport, although slowly, through the packaging film. The critical limit of 0.6 for water activity of cacao beans was not reached. So, no mould development because of storage is to be expected.

4. Moisture content

The moisture content of the beans was measured in duplicate from one sample at the surface and one sample from the middle of the vacuum package. In Figure 2 the evolution of the moisture content is illustrated over the storage period.

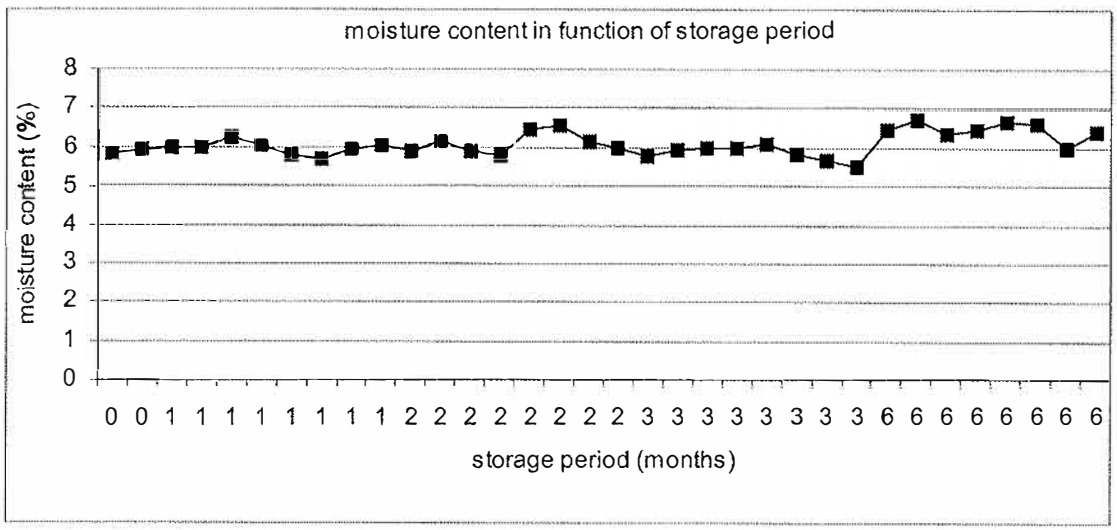

Figure 2. Moisture content (%) of the cacao beans in function of storage period, stored in vacuum package, under extreme climate conditions (30°C and 80% RH)

In Figure 2 it becomes clear that the moisture content is increasing in month 6 of the storage period. Out of the sorption isotherm for cacao beans it was concluded that a moisture content of 7% is agreeing with a water activity of 0.46 until 0.63 (microbiological unstable product). The water activity of the cacao beans stayed below the maximum water activity of 0,6 (Figure 1). Consequently, no microbiological proliferation was allowed during the 6 months of storage in the extreme climate conditions.

With the statistical program SPSS an independent T-test was done in order:

(1) to evaluate the difference in moisture content between the different months (month 1, 2, 3 and 6 were compared with the initial quality (month 0)) (= influence of storage period)
(2) to evaluate the difference between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 4** the average in moisture content is given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 4. Statistical interpretation of the evolution of the moisture content (%) of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average moisture content	Statistical conclusion	Average moisture content of sample at the surface of the package	Average moisture content of sample at the middle of the package	Statistical conclusion
Month 0	5.88				
Month 1	5.95	No significant difference	5.86	6.05	No significant difference
Month 2	6.10	No significant difference	6.25	5.96	No significant difference
Month 3	5.85	No significant difference	5.90	5.80	No significant difference
Month 6	6.45	Significant difference	6.43	6.29	No significant difference

No significant differences could be demonstrated between month 0 (initial quality) and the months 1, 2 and 3. But for month 6 a significant increase in the moisture content was detected. Although an increase in moisture content and water activity no critical limits are obtained for the cacao beans, stored in the vacuum packages and at 30°C and 80% RH. These limits were defined with the water sorption isotherm as 0.63 water activity and a moisture content of 7.13 %. A moisture content of 7.3% was previously defined as reasonable for a long term preservation (Challot and Vincent, 1977).
Also between the samples, taken from the surface of the package and from the middle of the vacuum package, no significant differences in moisture content could be demonstrated. Same trend was found for the water activity (Table 3).

Conclusion: The barrier properties of the packaging film for water vapour is very high, in these extreme climate conditions, water vapour could transport, although slowly, through the packaging film. A significant increase in moisture content was detected in month 6, but the critical limit of 7.13% of moisture content of the cacao beans was not reached. So, no mould development because of storage is to be expected.

5. pH

Acidity of beans is influenced by several factors and fermentation method is known to be crucial, normally the pH of dried beans range from pH 4.6 to 5.6 (Bonaparte et al., 1998). The pH was measured according to the method described by Bonaparte et al. (1998).

In Figure 3, the evolution of the pH of the stored cacao beans is illustrated.

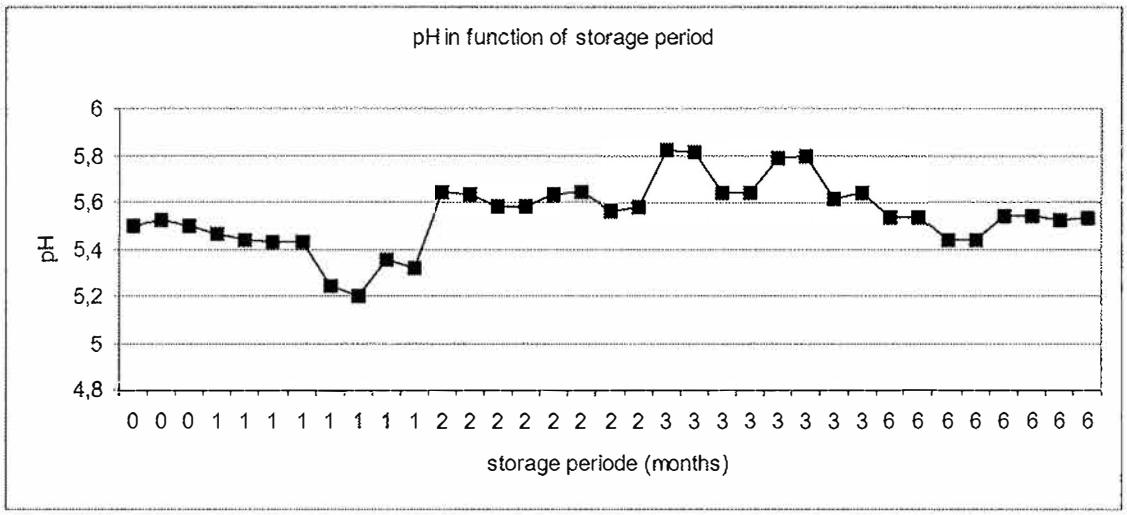

Figure 3. Evolution of the pH of cacao beans, in function of storage period. Cacao beans are stored in vacuum packages at 30°C and 80% RH.

With the statistical program SPSS an independent T-test was done in order:

(1) to evaluate the difference in pH between the different months (month 1, 2, 3 and 6 were compared with the initial quality (month 0)) (= influence of storage period)
(2) to evaluate the difference between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 5** the average of the pH are given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 5. Statistical interpretation of the evolution of the pH of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average pH	Statistical conclusion	Average pH of sample at the surface of the package	Average pH of sample at the middle of the package	Statistical conclusion
Month 0	5.51				
Month 1	5.36	Significant difference	5.33	5.38	No significant difference
Month 2	5.60	Significant difference	5.63	5.57	Significant difference
Month 3	5.72	Significant difference	5.80	5.63	Significant difference
Month 6	5.51	No significant difference	5.54	5.48	No significant difference

An increasing trend in pH was seen in the months 1, 2 and 3 but was not confirmed in month 6. Also between the pH of the samples taken at the surface of the package or in the middle of the package no differences or trends could be observed. The pH stayed stable during the storage of the cacao beans in the vacuum packages and at the extreme climate conditions.

Conclusion: the storage of the cacao beans in the vacuum packages and at the extreme climate conditions had no influence on the pH and consequently, taste and flavour properties of the beans.

6. Titratable acidity (TA)

Titratable acidity is a better measure of the total acids of cacao beans than pH, and both parameters have been correlated with taste scores or flavour acidity (Bonaparte et al., 1998). The method for determinating the TA of the cacao beans was described by Bonaparte et al., (1998).

Figure 4 shows the evolution of the TA value in function of the storage period.

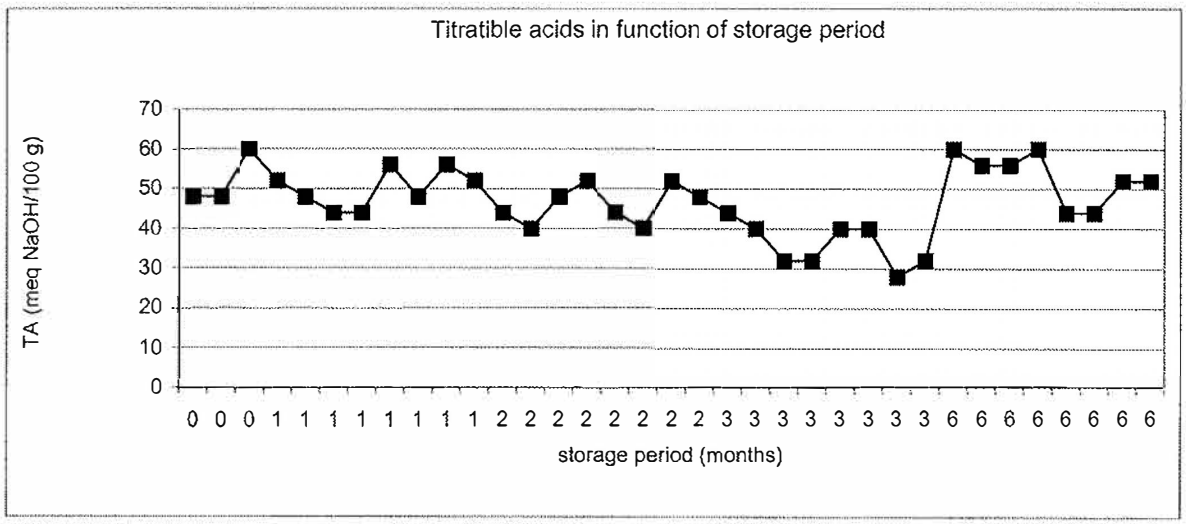

Figure 4. Titratible acids of cacao beans in function of storage period, stored in vacuum package at 30°C and 80% RH

The amount of TA varied during the storage, from sample to sample. No trend could be found. This result could be expected as the pH did not show a trend either during the storage period.

With the statistical program SPSS an independent T-test was done in order:

(1) to evaluate the difference in TA between the different months (month 1, 2, 3 and 6 were compared with the initial quality (month 0)) (= influence of storage period)
(2) to evaluate the difference in TA between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 6** the average of the TA are given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 6. Statistical interpretation of the evolution of the TA (meq NaOH/100g) of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average TA	Statistical conclusion	Average TA of sample at the surface of the package	Average TA of sample at the middle of the package	Statistical conclusion
Month 0	52				
Month 1	50	No significant difference	51	49	No significant difference
Month 2	46	No significant difference	42	50	Significant difference
Month 3	36	Significant difference	41	31	Significant difference
Month 6	53	No significant difference	51	55	No significant difference

As can be seen in Figure 4, the TA values are variating for the different analysed vacuum packages or samples but no systematic trend can be derived from Figure 4 nor from Table 6. The TA value did not change as a function of the storage period or as a function of the portion inside the vacuum packages.

Conclusion: the TA value was not influenced by storage period or by the extreme climate conditions in which the cacao beans were stored for 6 months. Consequelty, taste and flavour properties of the beans are not changed during the storage.

7. Peroxide value (PO)

The peroxide value is expressed as meq O_2/kg fat and was determinated by the titration method.

In Figure 5 the evolution of the peroxide value of the cacao beans is demonstrated in function of the storage period.

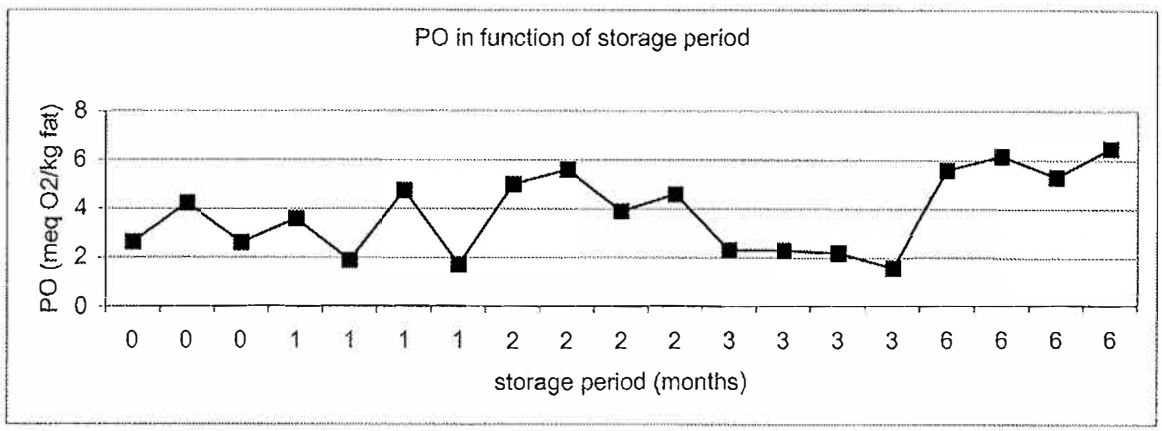

Figure 5. Evolution of peroxide value (meq O_2/kg fat) in function of the storage period, cacao beans are stored in vacuum packages at 30°C and 80% RH

The peroxide value is variating in function of the analysed sample: already a big variance is occurring in month 0 (initial quality). During the further analyses, variation was also found between the different samples. In month 6, however, higher values were measured for PO value.

With the statistical program SPSS an independent T-test was done in order:

(3) to evaluate the difference in PO between the different months (month 1, 2, 3 and 6 were compared with the initial quality (month 0)) (= influence of storage period)
(4) to evaluate the difference in PO between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 7** the average of the PO are given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 7. Statistical interpretation of the evolution of the PO (meq O2/kg fat) of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average PO	Statistical conclusion	Average PO of sample at the surface of the package	Average PO of sample at the middle of the package	Statistical conclusion
Month 0	1.94				
Month 1	2.98	No significant difference	4.17	1.80	Significant difference
Month 2	4.77	No significant difference	4.45	5.10	No significant difference
Month 3	2.1	No significant difference	2.15	1.95	No significant difference
Month 6	5.89	Significant difference	3.80	6.32	Significant difference

Out of the statistical interpretation of the PO values, it can be concluded that no significant difference can be found between the different storage months until month 6. Consequently, an influence of the storage period was detected on the PO value of the cacao beans. Also, between the samples, taken from the surface or in the middle of the packages in month 6, a significant higher PO value was analysed.

Conclusion: it can be concluded that the PO value increased during storage of the cacao beans in the vacuum packages, stored in the extreme climate conditions, between month 3 and month 6. Big variations were found between the different samples. Oxydation of the fat in the cacao beans started from month 6.

8. Free fatty acids

The amount of free fatty acids (FFA) is expressed as % and was determinated by the titration method.

In Figure 6 the evolution of the amount of free fatty acids of the cacao beans is demonstrated in function of the storage period.

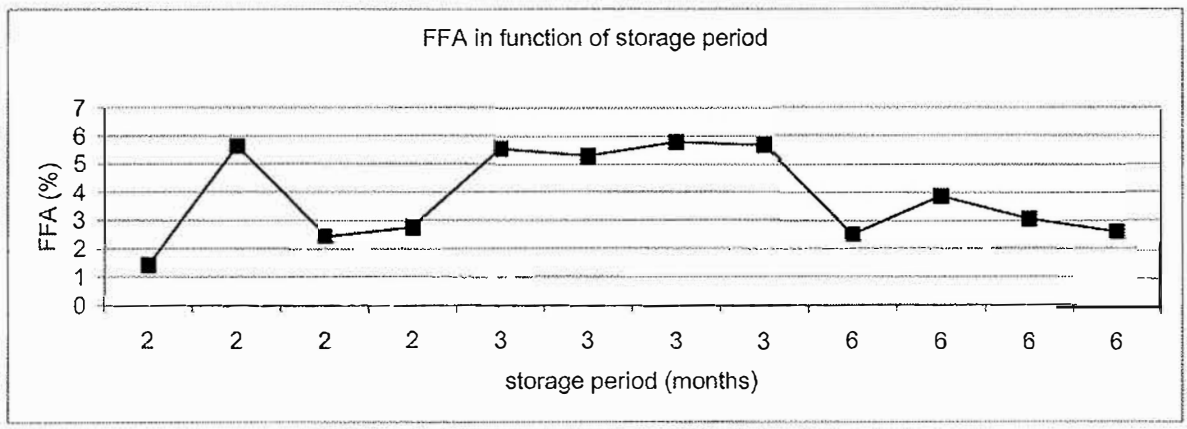

Figure 6. Percentage of Free Fatty Acids of cacao beans, stored in vacuum systems at 30°C and 80% RH, in function of storage period.

The amount of free fatty acids is increasing in month 3 compared to month 2 and 6. Again, as for the PO value, a big variation is detected between the different samples.

With the statistical program SPSS an independent T-test was done in order:

(1) to evaluate the difference in FFA between the different months (month 3 and 6 were compared with the month 2) (= influence of storage period)
(2) to evaluate the difference in FFA between the samples from the surface and the middle of the package (= influence of the storage conditions)

In **Table 8** the average of the FFA are given per month, and as well per sample (surface or middle). Also the result of the statistical analysis is indicated.

Table 8. Statistical interpretation of the evolution of the FFA (%) of cacao beans, stored in vacuum packages at 30°C and 80% RH

	Average FFA	Statistical conclusion	Average FFA of sample at the surface of the package	Average FFA of sample at the middle of the package	Statistical conclusion
Month 0	Not analysed				
Month 1	Not analysed				
Month 2	3.07		1.94	4.21	Significant difference
Month 3	5.59	Significant difference	5.68	5.50	No significant difference
Month 6	3.02	No significant difference	1.29	3.24	Significant difference

Between month 2 and 3 a significant increase was detected in FFA amount but this progression was not confirmed in month 6. The amount of FFA will increase will increasing moisture content in the cacao beans.

Conclusion: For the FFA value of the cacao beans no increasing or decreasing trend could be established during the storage experiment. The values of FFA had a variability between the different samples.

9. Final conclusion:

The gas composition of the headspace was stable and the vacuum of the packages was stable during storage for 6 months at extreme conditions (80% RH and 30°C). The barrier properties of the applied packaging film were strong enough to prevent substantial transport of gasses and water vapour.

No trend could be found in the different aspects of the visual quality (amount of violet beans, internal and external disorders, internal and external mould development, insect infestation) of the cacao beans during the six months of storage. A variation was detected between the different samples in their visual quality but no significant increase or decrease could be established.

The water activity of the beans increased during storage period but stayed below the critical value of 0,60. A similar conclusion can be made for the moisture content: an increase is detected during the storage period but the critical value for microbial growth of 7,13% (as was determinated by the sorption isotherm) was never reached.

The storage period and the extreme storage conditions (30°C and 80% RH) did not have an influence on the pH and the titritable acids. These parameters stayed more or less constant and consequently, no changes in taste and flavour can be expected.

The parameters for fat degradation, PO and FFA showed a high variability between different samples. Only, the PO value increased significantly in month 6 and can lead to differences in taste or other sensory properties.

It can be concluded, that the Vacuum Packaging System protects the cacao beans from quality degradation during long-term storage in extreme climate conditions. The microbial storability and safety of the product is assured as the water activity and the moisture content are staying far below their critical values in six months of storage. Towards, sensorial properties, no changes will be expected as the pH, TA (both determinating the taste and flavour properties) and PO (determinating the rancidity of the beans) are staying stable during storage.

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