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Application of Response Surface Methodology for Studying the Effects of Gestation and Post-Harvest Storage on the Shrinkage Characteristics of Pineapple cv. 'Smooth Cayenne' Fruits

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ABSTRACT

Response surface methodology was used to study the combined effect of gestation period and storage time on the shrinkage characteristics of pineapples fruits during post-harvest storage. A 6 × 10 factorial experimental design was used to study the combined effect of gestation period (140, 144, 148, 152, 156 and 160 days after floral induction) and storage time (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days) on the shrinkage evolution, shell colour break and crown withering index. Regression models were developed to predict the variables and their effects on the studied indices. Gestation period and storage time showed varied influences in dictating the shrinkage characteristics of pineapple fruits. Increasing storage time caused consistent and significant increases in the levels of shrinkage evolution, shell colour break and crown withering index during the 10-day storage period. Shrinkage evolution and shell colour break were not influenced by the gestation period (days of harvest after floral induction [FI]) irrespective of storage time. On the contrary, crown withering index was influenced by gestation period. Fruits harvested after 160 days at FI had higher mean crown withering index than those harvested after 140 days after FI, with the actual withering showing after 5 days of storage, suggesting that withering of pineapple cv. 'Smooth cayenne' fruits is influenced by both gestation period and storage time. Thus, response surface methodology could be employed to study the mass shrinkage characteristics of pineapple fruits as influenced by gestation period at FI and post-harvest storage time.

Keywords: *Ananas comosus*, crown withering, floral induction, gestation period, shell colour, shrinkage evolution

INTRODUCTION

The pineapple, *Ananas comosus*, belongs to the family *Bromeliaceae* and it originated from tropical America. The fruit was domesticated by the Indians and carried by them up through south and Central America to Mexico and the West Indies long before the arrival of the Europeans. Currently, pineapple is the world's most popular non-citrus tropical and subtropical fruit (Py *et al.* 1987; Paull and Chen 2003; Gil *et al.* 2006; Chonhenchob *et al.* 2007; Montero-Calderón *et al*. 2008; Rocculi *et al*. 2009; Hassan *et al.* 2010).

Storage of the fresh fruits prolongs their usefulness and improves their quality. It also checks market glut, provides wide selections of fruits throughout the year, helps in orderly marketing, increases financial gain to the producers and preserves the quality of the products (Soler 1992; Essuman 2003; Budu and Joyce 2005). The principal aim of storage is to control the rate of transpiration, respiration, disease infection and to preserve the commodity in its most usable form for consumers. To date, refrigeration is the only known economical method for long-term storage of fresh fruits and all other methods of regulating ripening and deterioration are at best only supplemental to low temperature (Swarts 1991; Hassan *et al.* 2010). In fact, other quality maintaining methods will not work satisfactorily without refrigeration. In a hot tropical climate, controlled atmosphere (CA) storage, waxing and use of polyethylene bags are not very effective if not combined with refrigeration as deterioration would be fast due to build-up of heat and CO_2 (Budu and Joyce 2005; Montero-Calderón *et al*. 2008; Rocculi *et al*. 2009).

The fragility of pineapple fruits calls for care in harvesting and subsequent handling and transportation in order to ensure that the fruits get to the market in a good condition. King (2002) asserts that inspite of their robust appearance; pineapples are very delicate and extremely susceptible to bruising and other mechanical injury. Premium quality fruits are obtained when the fruits are harvested at the optimum stage of development. The optimum condition for harvesting is a subjective operation which is market or consumer demand driven but economic limitations can play a determining role (Bartels 1996). When the fruits are picked unriped they never attain the fine appearance of fruits picked fully ripped. Immature fruits cannot be ripened always successfully artificially and it may have an acid taste and may develop a distinct yellow tinge (King 2002; Essuman 2003). An important criterion for judging when to harvest is the sugar content of the pineapple fruit (Py *et al.* 1987; Soler 1992; Ramsaroop *et al.* 2007; Rocculi *et al.* 2009). The sugar content increases very rapidly during the last stages of the gestation period of the fruits on the plant. Thus, the fruits should be harvested at the last stages of the gestation period taking into consideration the dictates of market demand. However, very little is known about how gestation and storage influence the shrinkage characteristics of the pineapples fruit during post-harvest handling and storage.

Response surface methodology (RSM) is a statistical-mathematical method which uses quantitative data in an experimental design to determine, and simultaneously solve multivariate equations to optimize processes and products (Giovanni 1983; Afoakwa *et al.* 2002, 2007, 2008). Thus, the objective of this work was to study the effects of gestation and post-harvest storage on the shrinkage characteristics of pineapple cv. 'Smooth Cayenne' fruits using response surface methodology.

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MATERIALS AND METHODS

Raw materials

Pineapple cv. 'Smooth Cayenne' fruits with known crop history were freshly harvested as intended for export from a major pineapple exporter (Jei River Farms, Kasoa, Ghana). They were sorted and graded into size, weight, shell, colour and crown conditions and transported to the laboratory for analysis.

The weighed fruits were placed in cardboard boxes in six (6) fruits/box as used for export and subjected to cold storage (8°C) at the Airways Catering Ltd., Kotoka International Airport, Accra, Ghana. The relative humidity was monitored daily and recorded to be between 82-85%.

Experimental design

The study was conducted using a 6 × 10 factorial experimental layout. The factors investigated were as follows:

i. Gestation period (140, 144, 148, 152, 156 and 160 days after floral induction);

ii. Storage interval (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days)

Dependent variables measured were as follows; shrinkage evolution (SE), shell colour break (SCB) and crown withering index (CWI). All the samples were analysed in triplicates and the mean values reported.

Methods

The shrinkage evolution of the pineapples was determined as follows: Mass shrinkage loss (S_L) was defined as weight loss by pineapple due to natural water loss. The ratio of decrease in weight (W) to initial weight (weight were measured using a Sartorius scale model 9100). Thus, the shrinkage evolution was:

$$\% S_L = [(W_I - W_F) | W_I) \times 100]$$

where W_I = initial weight reading; W_F = final weight reading.

The shell colour break of the fruits was compared with the standard chart for West Africa (SCWA) pineapples with the following grades: 1 = 1/8 shell ripe (yellow); 2 = ¼ ripe; 3 = ½ ripe; 4 = ¾ ripe; 5 = whole shell ripe; 6 = over ripe (dark brown patches).

CWI was evaluated by rating on a 1–3 scale (Soler 1992), where 1 = firm, dark green, fresh leaves; 2 = limp, some leaves brown (mostly basal leaves); 3 = dry, flaccid and most leaves brown (more than half of crown)

Statistical analysis

The data obtained from the experiments were statistically analyzed using Statgraphics (Graphics Software System, STCC, Inc., Rockville, MD, USA). Comparisons between sample treatments and the indices were done using analysis of variance (ANOVA) with a probability $P \leq 0.05$. Duncan's multiple range test was employed to compare mean values when the significant variance was found by ANOVA. A stepwise multiple regression analyses was conducted on the data to relate gestation period and storage time to shrinkage evolution, shell colour break and crown withering index. Regression models were developed to predict the variables and their effects on the studied indices. The models generated were plotted and presented as 3-dimensional plots in the function of 2 factors (gestation period and storage time). The adequacies of the fitted models were evaluated using the test of lack-of-fit, F-value and R^2 values. A good fitted model's R^2 value should be at least 80% but for a preliminary study an R^2 value of 60% is acceptable (Joglekar and May 1987; Malcolmson *et al.* 1993).

RESULTS AND DISCUSSION

Shrinkage evolution

The mass shrinkage characteristics of pineapples are of great significance to the post-harvest management of the fruit for export. Apart from its contribution to quality deterioration, it influences the overall returns for economic export, its shelf-life and consumer appeal (Dixie 1995; Essuman 2003).

Table 1 ANOVA summary table showing F-values of parameters evaluated.

Sources of Variation	Shrinkage Evolution	Shell Colour Break	Crown Withering Index
Gestation (G) days	45.00	65.06	1000.01
Storage time (ST) days	1000.00	80.34**	1000.00
G x ST	1000.00	1.79	1000.00

* - Significant at P ≤ 0.05; ** - Significant at P ≤ 0.01

Table 2 Summary of means (DMRT (LSD) * for parameters + significantly affected by treatment.

Treatment	Level	Shrinkage Evolution	Shell Colour Break	Crown Withering Index
Gestation period	140	2.73 a	4.83a	1.62a
(days after FI)	144	2.76 a	4.86a	1.67a
	148	2.84a	4.94a	1.69b
	152	2.96a	4.98a	1.72b
	156	2.98a	5.01a	1.74b
	160	3.02a	5.05a	1.75b
Storage time	1	0.239a	3.37a	1.00a
(days)	2	0.904b	3.63a	1.00a
	3	1.537c	4.00b	1.00a
	4	2.137d	4.37c	1.00a
	5	2.775e	4.63c	2.00b
	6	3.419f	5.13d	2.00b
	7	4.050g	5.37d	2.00b
	8	4.695h	5.75e	2.25c
	9	5.381i	5.75e	2.25c
	10	6.093j	5.87e	2.25c

* Means with the same letters within the columns of a treatment are not significantly different at P ≤ 0.05
DMRT – Duncan's Multiple Range Test, G – Gestation, ST – Storage Time, FI – Flo

Table 3 ANOVA for full regression of shrinkage evolution showing lack-of- fit.

Source	SS	df	MS	F-ratio	P-value
Total	294.442	79			
Model	293.665	5	58.733	5596.92	0.0001
Error	0.77654	74	0.0104		
Lack of fit	0.333648	34	0.0098	0.8852	
Pure error	0.442894	40	0.0117		

The model generated to predict shrinkage evolution could explain over 99.7% of variation within the model. Storage time alone could explain 93.0% of the variation, indicating that only 8.7% was due to the effect of gestation period (**Tables 1, 2**). Analysis of variance (ANOVA) on the model (**Table 3**) showed that the effect of storage time was highly significant ($P \leq 0.01$), with an insignificant lack-of-fit for the model. Further ANOVA showed that with the exception of gestation period, all the variables within the model were significant (**Table 4**). The regression equation obtained for the shrinkage evolution of the fruits is:

$$Z = 0.0141 - 0.367X_1 + 0.28X_2 + 0.0037X_2^2 + 0.210X_1X_2$$

where Z = shrinkage evolution, X_1 = gestation period; X_2 = storage time; R^2 = 0.9977.

The response surface plot (**Fig. 1**) indicates that shrinkage increased consistently with increasing storage time and no variation was observed with the gestation period. Analysis of variance of the data (**Table 1**) showed that the storage time were highly significant in influencing the shrinkage evolution of the pineapple. The contribution of storage time to shrinkage as revealed by mean separation (**Table 2**) showed that mean shrinkage increased with increasing storage interval. Mean shrinkage was least (0.329%) on day 1 and highest (6.093%) on day 10.

On the other hand, the response surface plot (**Fig. 1**) indicated that shrinkage showed no variation with gestation

Table 4 Model fitting results showing coefficients of the variables.

Variable	SE	R^2	SCB	R^2	CWI	R^2
B_0	0.141	-	0.700	-	2.033	-
X_1	-0.367*	0.002	-	-	0.525 *	0.0122
X_2	0.287**	0.93083	0.125 **	0.78120	0.454 **	0.85424
X_2^2	0.0037**	0.0002	-	-	-0.014	0.07857
X_1X_2	0.210	0.06561	0.035 *	0.03566	-	-
R^2	-	0.9976	-	0.8168	-	0.9453
F-ratio	-	5596.92**	-	82.52 **	-	207.407 **

B_o – Constant; X_1 – Gestation Period (Days); X_2 – Storage Time (Days); SCB - Shell Colour Break; CWI – Crown Withering Index
* - Significant at $P \leq 0.05$; ** - Significant at $P \leq 0.01$

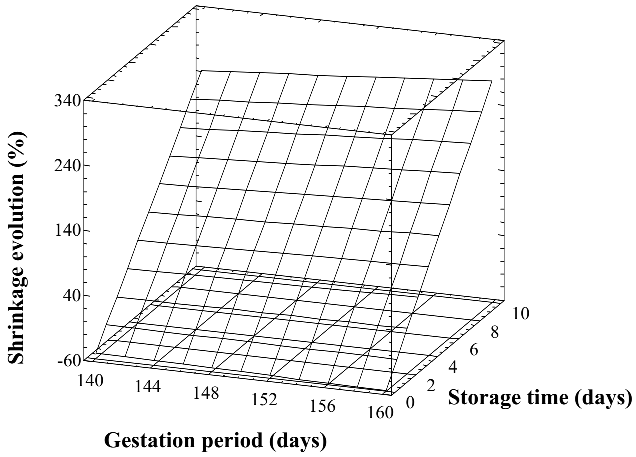

Fig. 1 Effect of gestation period and post-harvest storage on the shrinkage evolution of pineapple cv 'Smooth Cayenne' fruits.

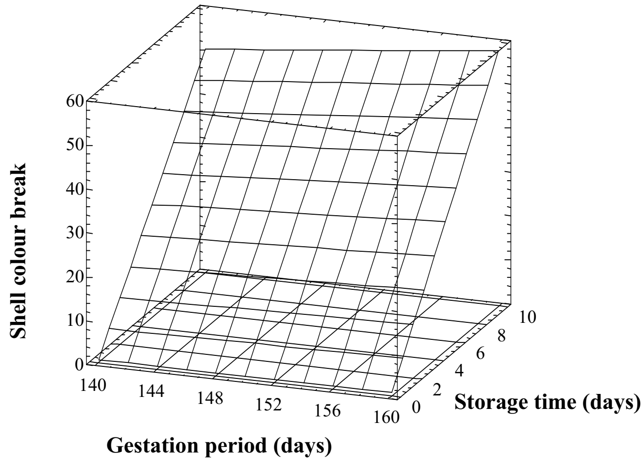

Fig. 2 Effect of gestation period and post-harvest storage on the shell colour break of pineapple cv 'Smooth Cayenne' fruits.

period. This means that fruits harvested between 140 and 160 days of floral induction behaved similarly with respect to their shrinkage evolution. Analysis of variance of the data (**Table 1**) showed that the gestation period was insignificant ($P > 0.05$) in influencing the shrinkage evolution of the pineapple. However, mean separation by multiple range test (**Table 2**) further revealed that the mean shrinkage evolution in fruits harvested at 160 days after floral induction (FI) was slightly higher (3.02%) than fruits harvested at 140 days FI (2.73%) but the observed differences were insignificant ($P > 0.05$). This observation explains that there is a potential risk of shrinkage from prolonging the harvest day beyond 140 days FI even though their difference is statistically insignificant. Pineapple is known to exhibit Crassulacean Acid Metabolism (CAM), a physiological phenolmenon of epiphytes and xerophytes (Kluge and Ting 1998). In CAM plants, there is a high metabolic rate occurring after three months of its floral induction with their stomata opening at night and close during the day to allow assimilation of CO_2 from the atmosphere (Bartels 1996). This mechanism allows for more water loss during the period with enhanced shrinkage, which accounts for the slightly increasing shrinkage evolution after 140 days.

Shell colour break

Pineapple shell colour is probably the most important quality attribute for consumer acceptance. It is no wonder that numerous quality standards involving shell colour have emerged from the world pineapple trade (Py *et al.* 1987). King (2002) reports that shell colour was about 75-100% deep yellow or orange shade and very little or no blemish is considered excellent in the world market for pineapples. Analysis of variance of the data (**Table 1**) showed that SCB was significantly ($P \leq 0.05$) influenced by the storage time, with an insignificant effect by the gestation period. There was however a significant interaction among all the treatments.

The mathematical model generated using the stepwise regression technique could explain over 81% of variation within SCB (**Table 3**). The storage time contributed 78% of the variation in the model indicating that only 3% of the variation was due to the effect of gestation period after floral induction. Thus, the model depended significantly on the storage time but the interaction between the gestation period and storage time was also significant. Analysis of variance of the model showed that the model was significant at $P \leq 0.05$ (**Table 4**) with an insignificant lack of fit. The regression model obtained for predicting the shell colour break is given by:

$$Z = 0.700 + 0.125X_2 + 0.035X_1 X_2$$

where Z = SCB, X = gestation period; X = storage time; R^2 = 0.8169.

The response surface plot (**Fig. 2**) showed that the shell colour break of the fruits were accelerated consistently with increasing storage time and fruit harvested at 160 days after FI were prone to breaking faster than fruits harvested at 140 days FI. Prolonging the storage time as revealed by mean separation (**Table 2**) accelerated SCB irrespective of the gestation of the fruit (**Fig. 2**). During the maturation and storage of many fruits, there is change in fruit colour from green to orange or red. This change in is attributed to the loss of chlorophyll and the unmasking and synthesis of carotenoids with time (MacKinney 1981). Multiple range tests to evaluate the effect of gestation on SCB (**Table 2**) showed that harvesting fruits at 160 days FI gave a higher SCB (3.52) than harvesting fruits at 140 days F1 (2.73). This suggests that the shell colour break is also influenced by the gestation period, even though the differences observed were insignificant ($P \leq 0.05$).

Crown withering index

For the fresh fruit market the condition of the crown is considered as an important quality characteristic of pineapple as it dictates the freshness of the fruit (Essuman 2003). Kay (1985) also reported that crown leaves that are turgid and bright green with a good shell colour attract premium price. However, leaves turning brown at the edge or tip detract from the accepted appearance of the fruit and usually reduce its market value.

The model generated to predict the CWI could account

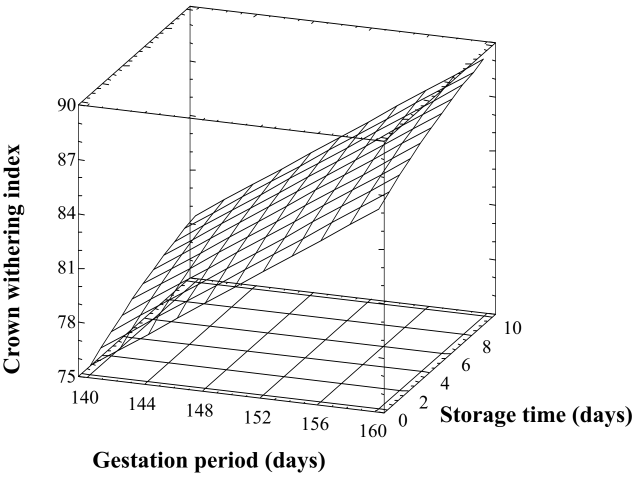

Fig. 3 Effect of gestation period and post-harvest storage on the crown withering index of pineapple cv 'Smooth Cayenne' fruits.

for over 94% of variation within the model. Again storage time alone could account for over 85% of this variation, indicating that only 9% was due to the effect of the gestation period. However, there was a significant contribution to the model by the gestation period and the quadratic term of the storage time (**Table 3**). Analysis of variance of the model showed that the model was highly significant ($P \leq 0.01$) in predicting CWI (**Table 4**), with an insignificant lack of fit. The regression model obtained for predicting the CWI is given by;

$$Z = 2.033 + 0.525X_1 + 0.454X_2 - 0.014X_2^2$$

where Z = CWI, X_1 = gestation period, X_2 = storage interval; $R^2 = 0.9453$.

The response surface plot (**Fig. 3**) indicates that CWI increased consistently with both increasing storage time. Increasing the gestation period from fruits harvested at 140 days of FI to 160 days after floral induction caused consistent increases in crown withering (**Fig. 3**). Likewise, increasing storage time led to consistent increases in the CWI. This means that both the storage time and gestation period influenced the CWI of the fruits.

ANOVA on the data showed that the CWI was significantly influenced by the gestation period and the storage time (**Table 1**). There was also a highly significant interaction between all the independent variables in the study. Means separation for the effect of gestation on the CWI revealed that fruits harvested at 160 days of FI had a higher mean CWI (1.75) than fruits harvested at 140 days FI (1.6). Thus suggesting that the longer the fruits are kept on the field after FI the greater the propensity towards crown withering after harvest. Mean separation for the effect of storage interval (**Table 2**) revealed that the actual withering of crown leaves was noticeable only after 5 days of storage where a CWI of (2.0) was noticed. This condition remained the same up to 7 days before yet another noticeable change on day 8 (2.25), which was maintained throughout the rest of the storage period. Thus, irrespective of the storage maturity (i.e. harvesting between 140 days FI and 160 days FI) pineapple fruit has an inherent ability to maintain the freshness of the crown up to about 5 days after harvest under ambient temperature (28 ± 2°C). This ability probably can be attributed to its xerophytic ontogeny.

CONCLUSION

Gestation period and storage time showed varied influences in dictating the shrinkage characteristics of pineapple fruits. Increasing storage time caused consistent and significant increases in the levels of SE, SCB and CWI during the 10 day storage period. SE and SCB were not influenced by the gestation period (days of harvest after FI) irrespective of storage time. On the contrary, crown withering index was influenced by gestation period. Fruits harvested after 160 days at FI had higher mean crown withering index than those harvested after 140 days after FI, with the actual withering showing after 5 days of storage, suggesting that withering of pineapple cv. 'Smooth cayenne' fruits is influenced by both gestation period and storage time. Thus, response surface methodology could be employed to study the shrinkage characteristics of pineapple fruits as influenced by gestation period at FI and post-harvest storage time.

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