DELAYING THE RIPENING OF BANANAS TO PREVENT SPOILAGE BY MEANS OF 1-METHYL CYCLOPROPENE

BY

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ABSTRACT

In order to determine the effectiveness of 1-methyl cyclopropene (1-MCP) in delaying ripening of harvested and mature desert bananas and hence reducing spoilage of banana, experiments were conducted using a total of 300 fingers of banana. Fingers were treated with concentrations of 1 ppm and 200 ppm of the 1-MCP, under normal and under controlled atmospheric conditions. The overall results indicated that there was an average of 2 days shelf life increment by using the 1-MCP at the concentration of 1 ppm when the application was done during the early ripeness stages where the mature bananas were still totally or almost totally green. In addition, the overall results indicated that there was an average shelf life increment of 3 days when the 1-MCP was applied at the same concentration of 1 ppm and then storing the bananas in air tight chambers. There was no significant effect when the 1-MCP was applied under normal atmosphere when the bananas were on the ripeness stages 4th or 5th and onwards, where beyond stage 7 of ripeness the bananas would be declared rotten. The 200 ppm of 1-MCP applied under normal atmosphere when the bananas were on their 4th and 5th stage of ripening shortened the shelf life by one day when fingers of bananas were detached from the hands. Moreover, 1-MCP was significantly effective ($p < 0.05$) in prolonging the shelf life of the banana mainly with fingers on the hand. So, it was determined that in order to retard ripening of banana more effectively by using 1-MCP, the concentration of 1-MCP should be about 1 ppm and with the banana fingers on hand and stored in closed containers soon after the application of the 1-MCP.

DECLARATION

I, Abdallah R. Mkanda, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Oct. 7, 2011

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The above declaration confirmed

7/10/2011

Prof. J.K. Mugula Date

(Supervisor)

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LIST OF ABBREVIATIONS AND SYMBOLS

$\mu l.l^{-1}$	-	Micro-litre per litre
1-MCP	-	1-methylcyclopropene
ANOVA	-	Analysis of Variance
h	-	Hour(s)
$nl.l^{-}$	-	Nano-litre per litre
^{o}C	-	Degree Celsius
pH	-	Hydrogen ion concentration
ppb	-	Parts per billion
ppm	-	Parts per million

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Agricultural loss has been known to significantly reduce the overall food availability to the public and adversely affect the economic welfare of farmers, farm crops retailers, and the general public in most developing countries. One of the biggest areas of this loss is through spoilage of some of the harvested produce (Simmonds, 1982).

The agricultural loss due to spoilage in developing countries is aggravated by poverty and technological backwardness, which make preservation methods that require the use of electric energy, or otherwise expensive methods, impractical. The affordable and available storage methods are not efficient enough to effectively prevent spoilage.

Of the crops grown in the farms, vegetables and fruits spoil the fastest. While fruits and vegetables in combination with grains provide the largest source of energy and nutrients to the people, and as well as being a common source of income to most small scale farmers and produce retailers, fruits spoilage alone has a significant share of the total farm crops loss (Robinson, 1996).

One very nutritionally important fruit which has achieved greater importance as cash and/or subsistence crop in most tropical areas is dessert banana (*M. Sapientum*). The tropical production areas of banana include Latin America, Caribbean, Philippines, and Africa. The bananas are also produced commercially in sub-tropical and Mediterranean climates such as Italy and Greece (Simmonds, 1982).

Bananas are eaten raw (uncooked) in their ripe state, in which they are sweeter and easily digested. Bananas are distinguished from plantains as plantains (*M. paradisiaca*) are starchier and are most often eaten as cooked while unripe. Still, however, there is considerable overlap between bananas and plantains in respect of the ways they are consumed (Robinson, 1996).

The dessert banana is nutritional superior to many other fruits; it has 75% of the pulp mass as water, large amount of potassium (K), and smaller amounts of calcium (Ca), phosphorus (P), magnesium (Mg) and sulphur (S). The pulp of ripe banana is also a good source of vitamin C (it has about 10 mg per 100g of pulp fresh mass ascorbic acid), vitamin A, thiamine (B_1), riboflavin (B_2), pantothenic acid, and pyridoxine (B_6) (Robinson, 1996). Also the pulp of banana contains about 23% carbohydrates, 1% protein, and 0.5% fat and is a good source of fibre (Chia, 1981).

Carbohydrates, in the pulp of the ripe banana, are present in simple sugars, mainly of three types: sucrose, glucose, and fructose. Banana is thus a good source of energy in comparison to many other fruits; a medium sized banana contains about 280 kilojoules, which is more than deciduous or citrus fruits (Simmonds, 1982).

The nutritional importance of bananas can also be appreciated from their therapeutic value. Ripe mashed banana is an excellent food for babies from the age of three months due to its easy digestibility and its mineral and vitamin content. It is good for the elderly as it can be consumed in large quantities without being fattening or causing digestive disturbances, is useful in managing patients with high blood pressure and heart disease as is low in sodium, contains very little fat and no cholesterol, is ideal for patients with gouts or arthritis as is free from substances that give rise to uric acid, and is ideal for diet of obese people due to the low lipid/high palatability combination (Robinson, 1996).

Also according to Morton (1987), antifungal and antibiotic principals are found in the peel and pulp of fully ripe bananas. The antibiotic acts against *Mycobacterium*; a fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants.

The fruit can also be processed for a number of food products. Ripe fruits can be pulped for puree for use in a variety of products including ice cream, yogurt, cake, bread, nectar, and baby food. Ripe bananas can be dried and eaten, or sliced, canned with syrup, and used in bakery products, fruit salads, and toppings. Green (unripe) bananas can be sliced and fried as chips. Whole green fruits can also be dried and ground into flour. Vinegar and alcoholic beverages can be made from fermented ripe bananas (Chia, 1981).

While an effective prevention or reduction of dessert banana spoilage would be just one small area in agricultural loss alleviation, it would yet be significant and important as apart from increasing the welfare of the banana producers and retailers, it is a component of an entire effort to minimize agricultural loss.

Spoilage of fruits in general, may be due to factors such as microbial, which is most commonly exhibited by fungal attack, or enzymatic, which is most likely to occur on cut fruits (Vieira, 1999). Where as causes and the nature of fruits spoilage form a very broad topic, ripening has been observed to encourage and hasten banana spoilage. A harvested banana fruit passes through three physiological development stages namely the pre-climacteric or 'green life' stage, the climacteric and ripening stage, and finally the eat-ripe and senescence stage (Robinson, 1996).

The pre-climacteric stage represents the period from harvest until initiation of respiratory climacteric. It is a period of low metabolic activity and the commercial objective is to prolong this period for as long as possible. This has been achieved by good pre-harvest management, by harvesting at an early stage of fruit maturity, and by low temperature control (13 °C) during transport of packed fruits (Vieira, 1999).

Sealed polyethylene bags, which have the effect of reducing water loss, reducing oxygen concentration, and increasing carbon dioxide concentration, all of which extend the pre-climacteric by reducing respiration rate and inhibiting ethylene synthesis, have been used to prolong the pre-climacteric stage of produce (Vieira, 1999).

The climacteric and ripening stage is characterized by a rapid and massive increase in ethylene production preceding the respiratory climacteric. This is a characteristic feature of climacteric fruits such as the banana. After triggering the initial rise in respiration, ethylene production decreases again. Ethylene is produced internally via the following pathway:

Methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene (Robinson, 1996).

The two enzymes that regulate ethylene synthesis in fruits are ACC synthase which generates ACC and ethylene forming enzyme (EFE) which generates ethylene from ACC. Once initiated, the climacteric is irreversible. The respiratory climax is identified by rapid oxygen uptake and carbon dioxide evolution to a maximum rate of 250 mg CO_2 $Kg^{-1}h^{-1}$ from a pre-climacteric low of around 30 mg CO_2 $Kg^{-1}h^{-1}$ (Robinson, 1996).

Ripening involves noticeable changes such as tissue softening, and the peel colour changes, normally from green to yellow or red, although some bananas ripen while remaining green. Just like for other fruits, during softening in the course of ripening, starch is degraded to sugars in both pulp and peel, and rupture strength of the cell walls slowly deteriorates. The concentration of soluble pectic polysaccharides and uronic acid, and their related enzymes activity, increases. The peel changes to yellow because the chlorophyll is broken down, and the pulp tastes sweeter since the ratio of sugars to starch increases, and a characteristic aroma is produced. Eventually the peel becomes spotted brown and then completely brown and the pulp loses its firm white texture to become brown and gelatinous (Robinson, 1996).

In the retail trade there is a colour chart for ripening bananas which has seven stages: the first stage represents a hard green fruit with high starch content while the fourth stage onwards exhibit colour development (the fruit more yellow than green), where at the end of seventh stage the fruit is soft yellow with brown flecks and high sugar content (Serek *et al.*, 1994).

Thus ripening is synonymous to aging: in addition to making the fruit more palatable, it expedites spoilage. It follows then, delaying of ripening would be an effective way of retarding fruit spoilage.

Researches have been undertaken to discover, create, or generate a ripening inhibitor which can be practically applied on the fruits to delay ripening. One of the most outstanding outcomes of the researches was the discovery and generation of 1-methyl cyclopropene (1-MCP) as a ripening inhibitor. The compound has the formula C_4H_6, and is basically a plant growth regulator (Blankenship and Dole, 2003).

The 1-MCP is an inhibitor of ethylene perception that can markedly affect ripening and/or senescence processes of many horticultural products; it is thought to act by binding irreversibly to ethylene receptors, rendering the crop insensitive to ethylene exposure (Blankenship and Dole, 2003). This binding results in fruit that maintains firmness and acidity levels higher than untreated fruit.

Most of the other effective ripening inhibitors such as aminooxyacetic acid and aminoethoxyvinylglycine were found to be toxic to tissue fruits and expensive (Bai *et al.*, 2003). Methyl bromide also has been known to inhibit ripening but at higher concentrations than 1-MCP; some types of fruits exposed to higher concentrations of methyl bromide have been known to exhibit severe phytotoxic response (Brecht *et al.*, 1985).

However, 1-MCP has been reported to have a non-toxic mode of action, a negligible residue, and to be active at very low concentrations. It has been shown to effectively delay the ripening of various fruits such as plums harvested at their second stage of ripening (Valero *et al.*, 2003), guava fruits (Singh *et al.*, 2003), etc. It has also been known to be effective in retarding repining of ethylene treated bananas that had originally not yet reached stage 3 of the ripeness stage colour chart (Pelayo *et al.*, 2002).

In the USA and Canada, 1-MCP is marketed as Ethyl Bloc TM (Floralife, Inc.) and Smart Fresh TM (Agro Fresh, Inc., Rohm and Haas); they are used on ornamental and edible horticultural products, respectively. All global use rights, however, are owned by Agro Fresh (Blankenship and Dole, 2003).

Food use registration for 1-MCP has been obtained in Argentina, Australia, Austria, Brazil, Canada, Chile, Costa Rica, Guatemala and Honduras, Israel, Mexico, The Netherlands, New Zealand, South Africa, UK, and the USA. Registered crops, which are specific to countries, include apple, apricot, avocado, kiwi fruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum, squash, tomato and tulip bulbs (Blankenship and Dole, 2003).

Commercial utilization of this technology is a function of the cost of 1-MCP application relative to its benefits for each product. The cost/benefit ratio is affected by many factors including the product response, especially in relation to quality as perceived by the consumer, how successfully 1-MCP use can be incorporated into handling, storage and transport systems, scale of the industry involved, competition in the marketplace and whether it provides market access to markets not available using current technologies.

In this study, the practicality, feasibility, and efficacy of applying the 1-MCP on desert bananas to delay ripening and hence reduce banana spoilage, in Morogoro, was investigated.

1.2 Problem Statement and Justification

In Morogoro, Tanzania, the spoilage of dessert bananas due to over ripening is very common; fingers of spoiled bananas are commonly seen on retailers' tables. In this situation the retailers then desperately mark down their overripe or half spoiled bananas, hoping to at least sell some, but mainly ending up in throwing the bananas away after becoming totally spoiled. This minimizes their profit making capacity and reduces the availability of nutrients to the public. This is also just one example of the entire problem of spoilage due to over ripening.

In fact, in this study, a survey on the amount of bananas that normally spoiled due to over ripening on retailers' tables showed retailers suffered significant economical loss as a consequence. The survey was conducted in Morogoro using a questionnaire with respondents selected randomly and mainly from Kihonda, Mazimbu market, Morogoro Central Municipal market, etc.

At the same time, cold storage or other expensive preservation methods are impractical. Hence, an inexpensive method of reducing spoilage of bananas is urgently needed. An appropriate method seems to be use of a non toxic ripening inhibitor that can work under ambient temperatures. The study of effectiveness and suitability of this method should involve locally available equipments and material so as to simulate a real life situation with respect to the technological level and living standards in the region should it be put into practical application afterwards.

The inhibitor of choice was 1- MCP and the choice took into consideration its ability to be active at very low concentration (Valero *et al.,* 2003), its non toxic mode of action and its ability to slow down vitamin C loss in some fruits (Selvarajah *et al.,* 2001).

1.3 Objectives of Study

1.3.1 Main objective

The objective of this research was to investigate the effectiveness of 1 – MCP in delaying ripening of banana under ambient conditions as a method of reducing spoilage of banana.

1.3.2 Specific objectives

a) To determine the effectiveness of 1-MCP as a ripening retardant on mature dessert banana under normal and under controlled atmosphere conditions at ambient temperatures.

b) To determine the minimum effective concentration of 1-MCP required to prolong the shelf life of bananas.

CHAPTER TWO

2.0 LITERATURE REVIEW

The 1-MCP availability as a stable, complex formulation, has led to a surge of research and commercial interest around the world. According to the Wikipedia online encyclopaedia, there are two principal commercial uses of 1-MCP: maintaining the freshness of ornamental plants and flowers, and preventing the ripening of fruits. Under the brand name EthylBloc, 1-MCP was approved by the U.S. Environmental Protection Agency for use on ornamental crops. It is used with cut flowers, potted flowers, and bedding, nursery and foliage plants to prevent premature wilting, leaf yellowing, premature opening of flowers, and premature death.

Under the brand name SmartFresh, 1-MCP is used in the agriculture industry by growers, packers, and shippers to maintain the quality of fruits and vegetables by preventing or delaying the natural ripening process (Wikipedia, 2009). Moreover, 1-MCP is also being developed as a new crop protection technique. By spraying 1-MCP on growing field crops during times of stress, the crops may be protected from moderate heat and drought conditions (Wikipedia, 2009). Most published studies involved treatment of products with 1-MCP immediately after harvest in the laboratory. And for many products, partial control of ripening was more desirable than total control (Pelayo *et al.*, 2002).

For edible climacteric organs, lower concentrations of 1-MCP that would allow beneficial delays in ripening, while still allowing positive aspects of ethylene action to occur, was necessary. For pear fruits, for example, treatment with the same rate of 1-MCP necessary to control ripening of many apples cultivars (typically 1 $\mu l.l^{-1}$) could totally inhibit ripening. Thus, to obtain desirable eating quality, treatment with lower 1-MCP concentrations might be required (Ekman *et al.*, 2004).

The ripening inhibitor (1-MCP) gained popularity among researchers due to its promising efficiency in post harvest preservation techniques. Especially where it could be hypothesized to slow down and even completely halting further ripening in fruits that had already begun to ripen. Harris *et al.* (2000) hypothesized that there was a significant interaction between the effectiveness of 1-MCP and the maturity of bananas; they found that 500 nl l^{-1} of 1-MCP at 20 °C for 24 h was more effective in delaying ripening as maturity progressed from 71 to 156-/173 days after bunch emergence, although the absolute time for bananas to ripen decreased from 40 to 28 days, respectively, at 20 °C in a continuous air flow containing 0.1 ml l^{-1} of ethylene.

Huber and Jeong (2003) reported that 1-MCP was able to retard the ripening of tomato at the turning stage of development, indicating that 1-MCP was effective in slowing post harvest ripening even when applied after ripening had started. Consequently, they concluded that 1-MCP had potential to increase overall fruit quality by allowing fruit to ripen on the vine for an extended period.

Sisler and Serek (2000) tendered a report on 1-MCP inhibitory activities. The gaseous compound 1-MCP inhibited a range of plant responses to ethylene, including ethylene-induced ripening of bananas. The effectiveness of the inhibition is demonstrated by the very low dissociation constants for the 1-MCP-binding site complex.

Sherman and Lee (1985) reported the tomato fruit ripening inhibition by means of methyl bromide (MB) treatment. A key feature of MB inhibition of ripening in tomato appeared to be reduced sensitivity to ethylene; the same way 1-MCP seemed to work.

However, methyl bromide has also been known to inhibit the ripening at higher concentrations than 1-MCP (Brecht *et al.*, 1985). And like many other ripening inhibitors such as aminooxyacetic acid and aminoethoxyvinylglycine according to Bai *et al.* (2003), methyl bromide has been known to exhibit severe phytotoxic response. So considering other researches, 1-MCP appeared to be the best option as far as choices of fruits ripening inhibitors.

Apart from the above mentioned, many other ripening and growth retardants which seem to work the same way 1-MCP does have been known to be disadvantageous, especially for commercial use. A cyclic diolefin, 2, 5 –norbornadiene, strongly inhibited plants response to ethylene by preventing its binding. However the effect was not permanent as the inhibition was reversible (Sisler *et al.*, 1986). The silver thiosulphate complex (STS) proved to be potential pollutant because it contained silver (Serek and Reid, 1993) and the Diazocyclopentadiene (DACP) although inhibited ethylene binding and the inhibition was irreversible just as was with 1-MCP, it proved to be explosive and hence not suitable for commercial use (Serek *et al.*, 1994).

Obviously, 1-MCP may not produce the same effect at a particular concentration when applied on different types of fruits. And with regard to consumption of the treated fruits, thorough research will be required to precisely determine the optimum 1-MCP treatment levels and length of exposure. Huber and Jeong (2003) reports that 1-MCP has been approved for commercial use only with apples.

Banana has been described as a typical climacteric fruit with unique characteristics. It exhibits a sudden increase followed by a rapid decrease in ethylene production, resulting in a sharp peak at the onset of ripening, different from other climacteric fruits where a gradual increase of ethylene production in parallel with a respiratory climacteric rise occurs; in addition, most of the climacteric fruits belong to dicotyledonous plants, while banana belongs to the monocotyledon group (Inaba *et al.*, 2006). The authors reported in their research that ethylene production was determined in the pulp and peel tissues separately in the fruit treated with 1-MCP. Interestingly, 1-MCP applied to banana fruit after onset of the climacteric rise enhanced ethylene production in the pulp greatly while suppressing it almost completely in the peel.

In a study by Pelayo *et al.* (2002) which involved the use 1-MCP, it was observed that the 1-MCP was effective in retarding ripening of bananas at stage 2 (peel colour light green) and below while it was much less effective in delaying the ripening of bananas at stages 3 (peel colour more green than yellow) and stage 4 (peel colour more yellow than green), and hence concluded that the 1-MCP was 'too inconsistent' in delaying the ripening of partially ripened bananas.

The effect of 1-MCP on nutritional quality has not been studied thoroughly. However, 1-MCP slowed vitamin C loss in cold-stored pineapples (Selvarajah *et al.*, 2001) and in minimally processed lettuce and pineapples (Budu and Joyce, 2003). This is also another reason for considering 1-MCP of all other reported ripening inhibitors.

Relatively little was known about 1-MCP effects on disease incidence. Factors that influenced the effects of 1-MCP on disease development were likely to be specific to the product and its interaction with a pathogen. In general, reduced ethylene sensitivity could be beneficial against some pathogens but deleterious to resistance against other pathogens, such as in citrus where mould and stem end rots were inhibited and enhanced by ethylene, respectively (Mullins *et al.*, 2000).

Although registration for use of any chemical on edible products is a major obstacle, the effects of 1-MCP on fruit ripening suggest potential for the future commercial possibilities. It is possible that fruits may be triggered to ripen to optimum ripeness, and further ripening could then be halted by 1-MCP application. These and other strategies warrant investigation as the licensing and registration of this promising new post harvest tool proceeds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 1-MCP Application

The focus of this part was to investigate the effectiveness of 1 – MCP in delaying ripening of Mtwike banana. Mtwike banana was selected to be used in this study because it appeared to be more prevalent in the study areas than other varieties of dessert bananas.

In these experiments to determine the effectiveness of the 1-MCP, fingers were deliberately obtained of the same ripeness colour stage possible in order to facilitate the measurement of the effectiveness of the ripening inhibitor. Most of the experiments were done in the months of June, July and August of 2008 where the mean temperature was 22°C and the mean shelf life from the 3rd and 4th stage of ripening of untreated fingers under normal atmospheric conditions was 7 days.

The brand name of the 1-MCP product used in this study was Lupo Fresh (Syntake chemicals, China Mainland, 1100 Wuzhong Rd, Shanghai), with the packing of 100mL/bottle; in an aluminium bottle and active ingredient of 0.41%. For treatment purposes, 1-MCP is a gas and it has to be applied in an airtight room or chamber.

The method of applying 1-MCP (spraying in air tight chambers) so as to get the required concentration was according to the manufacturer's instructions as appeared on the 1-MCP bottles. The specification on the label of the container directed that each second of spray covered $2m^3$ at the concentration of 1ppm, thus 11 seconds of spray in a $22m^3$ chamber would provide a concentration of 1 ppm and 2 seconds of spray in a $0.02m^3$ container would provide a concentration of 200 ppm. The effect of the 1-MCP on the ripening of the bananas was determined by the colour of the peel, conversion of starch to sugar, pH, and firmness test using the procedure described by Dadzie and Orchard (1997).

3.1.1 Treatment

Several experiments were run using "Mtwike" banana grown from four different locations (Tangeni, Mkuyuni, Mzinga, and Matombo) in Morogoro. Replication, randomization and precision were taken into consideration when the experiments were run. In some of the experiments the fingers were detached from the banana hands and in some experiments the fingers were left attached on the hands.

There were about ten separate replications; in five successful replications the reading of some treatments results were spoiled and hence resulted in an unequal number of fingers for each different treatment conditions. However, the program used for calculation of mean effect of treatments (SPSS Inc., 1989-2003) used harmonic mean and hence circumvented the need of reducing the sample size by removing some of the fingers in other treatment situations so as to come down to the same number of fingers per treatment condition. In the treatments there were:

1. Determination of the effect of 1 ppm and 200 ppm of the 1-MCP under normal and controlled atmosphere on fingers of bananas detached from the hand, where 126 fingers were involved.

2. Determination of the effect of 1 ppm and 200 ppm of the 1-MCP under normal and controlled atmosphere on fingers of bananas on the hand, where 174 fingers were involved.

The treatments were done by spraying the 1-MCP in a chamber to give the required concentration. For the 1ppm, the 1-MCP was sprayed in 22 m^3 tightly closed chambers for 11 seconds. According to the manufacturer of the 1-MCP (Syntake chemicals, China Mainland, 1100 Wuzhong Rd, Shanghai), the specification on the label of the 1-methyl cyclopropene container directed that each second of spray covered 2m^3, thus the 11 seconds of spray provided a concentration of 1ppm. For the concentration of 200 ppm, the 1-MCP was sprayed in a 0.02 m^3 container for 2 seconds.

The use of these two opposite extremes of concentrations of the 1-MCP (1 ppm and 200 ppm) as opposed to gradation between the extremes was partly due to the technical difficulties in applying the 1-MCP at such gradation of concentrations and mainly because it served the purpose of determining its (1-MCP) effectiveness at lowers versus higher concentrations.

After spraying, the fingers of banana were left in the chambers for 24 hours before being transferred to another chamber for the case of controlled atmosphere or exposed to the normal atmosphere for the duration of the experiment. Of the total 300 fingers used in the experiments, 100 fingers were used as control, meaning, there were not treated with 1-MCP. The treatments were conducted in the months of May, June, and July 2008. The ambient monthly mean temperatures when these treatments were done ranged from 21 to 22°C.

3.1.2 Colour of the peel

The colour of the peel was determined according to the procedure described by Dadzie and Orchard (1997). The colour was noted from day 0 to the last day of storage and compared with the peel colour ripeness stage chart.

3.1.3 Starch content and peel colour stage using iodine and the standard colour chart

Starch was determined according to the procedure described by Dadzie and Orchard (1997). Iodine was applied on slices of banana fingers from the sample and compared to the starch and peel colour ripeness stage chart at 0 (zero) day of storage. The same procedure was repeated at the last day of storage.

3.1.4 The pH of the pulp juice

The pH of the pulp juice was determined according to the procedure described by Dadzie and Orchard (1997). Exactly 30 g of banana pulp at stage 3 (the fruit more green than yellow) and stage 7 (fruit is soft yellow with brown flecks) in 90 ml of distilled water was blended for 2 minutes and then filtered and then universal indicator paper was immersed in the filtrate and colour change was compared with the pH colour chart.

3.1.5 Pulp Firmness

The pulp firmness was determined according to the procedure described by Dadzie and Orchard (1997). Samples of banana fingers at the 3rd and 7th stage of ripeness were cut transversely at the midpoint and placed on a hard surface and the forces required to penetrate the 1 cm of the 3^{rd} and 7^{th} ripeness stage samples pulp tissue with a 8 mm probe hand held Effegi penetrometer were respectively recorded.

3.2 Statistical Analysis

Factorial Research Design was used, where there was an application of two different levels of the 1-MCP (1 ppm and 200 ppm) on the 'Mtwike' bananas and subjection of the bananas to a controlled atmosphere (air tight container) to determine the effect of both factors individually and collectively on the shelf life the bananas. Statistical test used was Factorial ANOVA where SPSS 12.0 version (SPSS Inc., 1989-2003) was used to analyze the data and compare the means of the effects (shelf lives) of the level of the test factors (applied inhibitor and controlled atmosphere) on the banana.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 The Effect of 1-MCP on Starch, Colour, pH and Firmness

4.1.1 Starch content

The average decrease in the starch content, as indicated by the Iodine test for Starch changing pattern of scale of 1-7 corresponding to peel colour stage, was slower by three days difference when the 1-MCP was used at the concentration of 1 ppm than when the bananas were not treated or when the 1-MCP concentration was 200 ppm. This shows that the concentration of 1 ppm was more effective than higher concentrations as starch content is a reliable indicator of ripening of fruits.

4.1.2 Peel colour

As shown in Fig. 1 below, the average change in the peel colour as indicated by the standard colour chart of 1-7 peel colour stage was faster for the untreated bananas and those treated at the concentration of 200 ppm by three days than bananas treated at the concentration of 1 ppm of the 1-MCP. This result corroborates the finding from section 4.1.1 on 1-MCP and starch content. It again shows that the concentration of 1 ppm was more effective than higher concentrations.

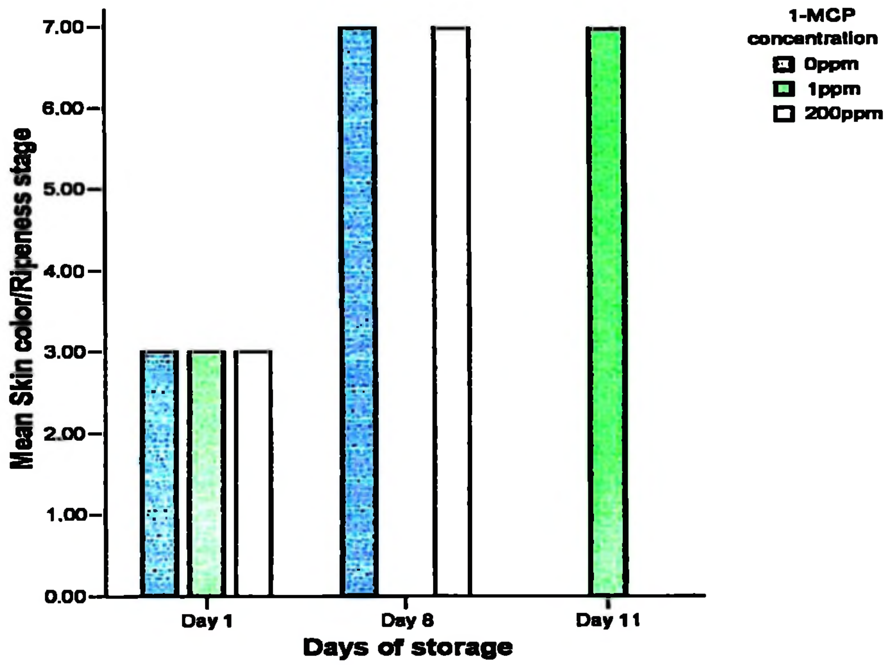

Figure 1: The effect of 1-MCP on the skin colour and ripeness stage of banana

4.1.3 Pulp pH

The pH in the 1-MCP treated and untreated banana fingers could not be clearly distinguished; neither could the pH of the first and last day of storage (The pH universal indicator gave a pH of about 6 in all situations). It could not be established whether the method used to determine pH was not sensitive enough to be effective or that there were no significant change in pH as the bananas were exhibiting peel colour and starch content change. Theoretically, the pH would be expected to increase (become less acidic) as fruits ripen.

4.1.4 Firmness

The decrease in the firmness of the bananas treated with 1 ppm of the 1-MCP was relatively delayed compared with the untreated bananas or those treated with the 1-MCP at the concentration of 200 ppm. The fingers of untreated bananas and those treated with 200 ppm of the 1-MCP reached the firmness of 0.9 N on the 8th day while those treated with 1 ppm of the 1-MCP reached the same average firmness of 0.9 N on the 11th day of storage (see Fig. 2 below). The skin colour of the bananas matched to the standard ripeness colour stage chart was used to determined the end of storage (stage 7 over ripe) for each sample with the different treatments which in turn (the storage end point) indicated the time for taking the last firmness measurement of a particular sample.

This delay in the lowering of the value of firmness when 1-MCP was applied at the concentration of 1 ppm suggested that the 1- MCP was more effectiveness at this concentration than the other concentrations used.

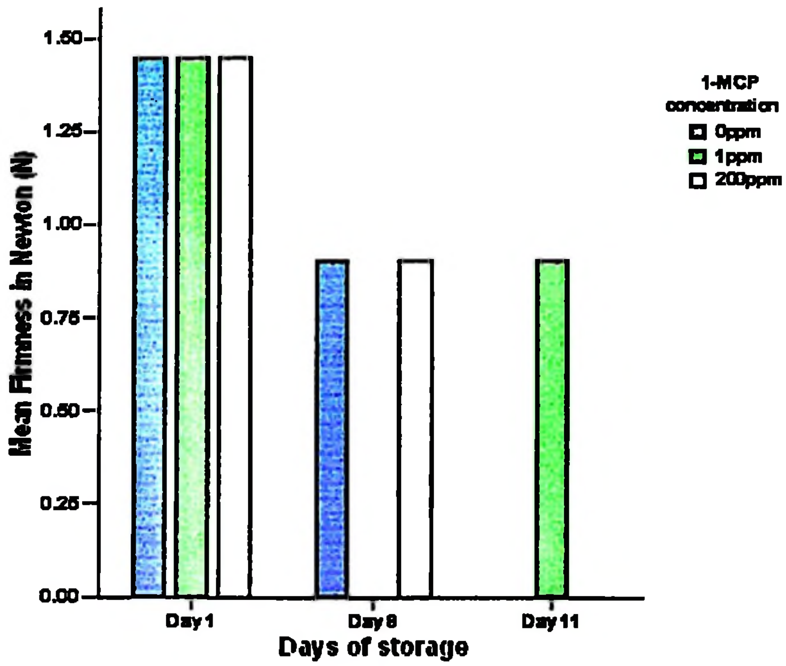

Figure 2: The effect of 1-MCP on the firmness of banana

4.1.5 Overall effect of the 1-MCP on shelf life

For the effect of the 1-MCP, factorial ANOVA using SPSS 12.0 version program gave the following results: The graph (Fig. 3) shows an average shelf life of about 11 days for the bananas treated with 1-MCP at the concentration of 1 ppm and an average shelf life of almost 8 days for the untreated bananas and those treated at the concentration of 200 ppm.

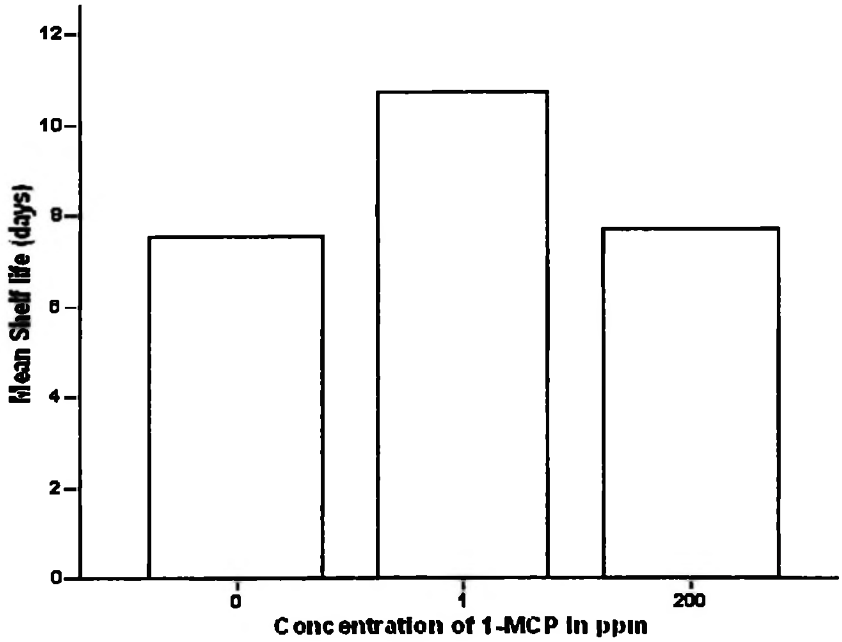

Figure 3: The effect of 1-MCP concentration on the shelf life of banana

The effect of 1-MCP concentration on the shelf life of banana was also as indicated in Table 1.

Table 1: The effect of 1-MCP concentration on mean shelf life of ripe banana

Concentration of 1-MCP (ppm)	Mean shelf life (days)
0	7.6± 0.2[c]
1	10.7± 0.3[a]
200	7.7± 0.2[b]

Also, controlled atmosphere by itself was found to have shelf life prolongation effect as shown in Table 2 below, where the shelf life under controlled atmosphere was found to be about 10 days while under normal atmosphere was 8 days.

Table 2: The effect of controlled atmosphere on shelf life of banana

Storage conditions	Mean shelf life (days)
Normal atmosphere	7.6± 0.2[b]
Controlled atmosphere	9.7± 0.2[a]

Generally, the main effect of the 1-MCP., The main effect of the nature of fingers (whether attached to the hand or not), the main effect of storage conditions, and the main effect of interaction between the concentration of 1-MCP and the storage conditions (whether confined in air tight chamber or exposed to normal atmosphere) were as indicated in Appendix 2.

It was generally found that: The main effect of 1-MCP was significant ($p< 0.05$). The overall mean shelf life at 1 ppm of 1-MCP was 10.7 (11 days); the overall mean shelf life at 200 ppm of 1-MCP was 7.7 (8 days), and the overall mean shelf life of untreated fingers was 7.5 (about 8 days).

The main effect of interaction between the concentration of 1-MCP and the state of fingers (whether detached or on hand) was significant. The mean shelf life was highest in detached fingers at both concentrations. The mean shelf life was prolonged and was higher at lower concentration in both detached and fingers on hand as shown in Table 3.

Table 3: The effect of 1-MCP on shelf life of detached and on hand fingers of banana

Fingers	Conc. of 1-MCP (p.p.m)	Mean shelf life (days)	
Detached	0	8.5	± 0.3^{b}
	1	13.3	±0.4^{a}
	200	8.0	±0.3^{c}
On hand	0	6.8	±0.3^{c}
	1	8.1	±0.4^{a}
	200	7.3	±0.2^{b}

The main effect of interaction between the concentration of 1-MCP and the storage conditions (whether controlled or normal atmosphere) was significant ($p< 0.05$). The mean shelf life was also higher at lower concentration in both detached and fingers on hand. The main effect of interaction between the concentration of 1-MCP, storage conditions and state of fingers was significant ($p< 0.05$). The mean shelf life was prolonged and was higher at lower concentrations in both detached and fingers on hand.

This study established that the 1-MCP and the controlled atmosphere factors had effect on the rate of ripening of the banana, where the application of 1-MCP at concentrations as low as 1 ppm extended the shelf life of Mtwike banana by 3 days (Plate 1).

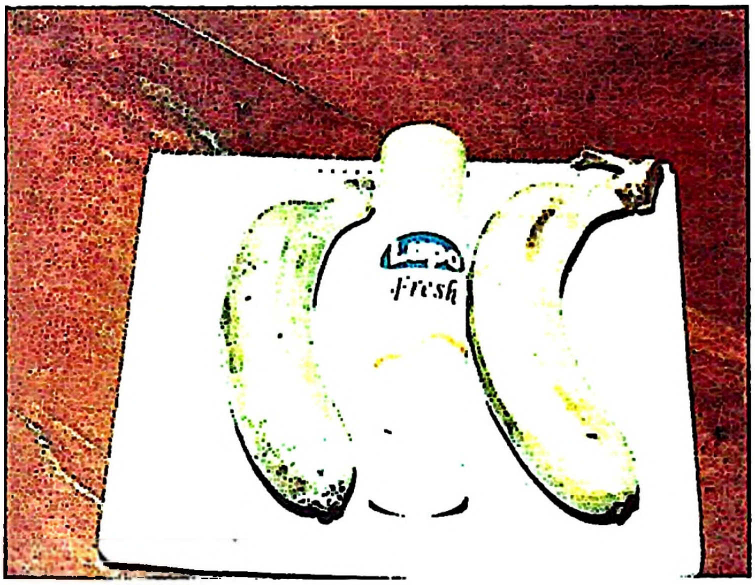

Plate 1: 1-MCP treated finger (left) and untreated finger (right) four days after application of the 1-MCP at the concentration of 1 ppm; they were originally obtained at ripeness stage 1.

In all of the experiments, the combination of 1-MCP and the controlled atmosphere led to increased shelf life; in all circumstances where the shelf life was increased as a result of combining 1-MCP and controlled atmosphere, the shelf life was most prolonged when the 1-MCP was as low as 1 ppm.

In a similar study, Sizler and Serek (2000), reported that 1-MCP effectively inhibited the ripening of bananas (which were exposed to ethylene to induce ripening) at a much lower concentration (in comparison to the one used in this study) of around 0.7

nl l^{-1} and the bananas regained sensitivity to ethylene again in 12 days at 25°C after treatment with 1-MCP. This suggests the possibility that 1-MCP is only or mainly effective at lower concentrations.

Higher concentrations of about 200 ppm of the 1-MCP under normal atmosphere on detached fingers of bananas appeared to have the effect of significantly shortening the shelf life (enhancing ripening); while for the fingers on the hand it appeared to have no significant effect.

In a similar experiment conducted by Pelayo *et al.* (2002) on the effect of 1-MCP on partial ripe bananas, it was concluded that the efficacy of 1-MCP in delaying the ripening of partially ripened bananas at the concentration of 1000 nl. l^{-1} was too inconsistent for commercial application. While in another research done on tomatoes and avocadoes fruit (Huber and Jeong 2003), it was observed that 1-MCP treatment resulted in nearly a doubling in shelf life of tomatoes and 'Simmonds' avocado fruit at storage temperature of 20°C and concentration of 400 ppb.

Causes of the observed deviations between the findings of this study and those of the others could be that different kind of fruits (tomatoes/avocadoes and bananas) and different species or varieties of the same fruit responded differently to 1-MCP. Also the concentration of 1-MCP could be a factor, where the concentrations used in this study were 200 ppm and 1 ppm while on the tomatoes and avocadoes (Huber and Jeong 2003) were as low as 400 ppb.

Dryness of the detached fingers might be one of the factors which led to the difference in the effect of the 1-MCP in this particular research. It was also observed that when the detached fingers were at the first or second stage of ripening they appeared to take longer to reach the 7^{th} ripening colour stage and looked much drier than fingers on hand under similar situations.

Also in this study, some of the fingers under controlled atmosphere had about 25% green colour on their peels at their 7^{th} colour ripeness stage, where as for this variety of banana species there would normally be at least 100% yellow with some brown spots. This could be because the peel and the pulp responded differently to the 1-MCP; the situation in this case might have been similar to the observation by Inaba *et al.* (2006), who reported in their research that ethylene production was suppressed in the peel and enhanced in the pulp. The ripeness stage of the bananas in this case (study) was established by means of combination of criteria which included the iodine test for starch content, pulp firmness and taste. Also, the detached fingers did not taste as good as fingers that were attached on the hand.

So, apparently the fingers lost much more moisture when detached than when were on the hand, and hence the metabolism of the ripening process was obviously slowed down due to low moisture content of the fingers.

When it came to the functioning 1-MCP, obviously it was impaired by the dry conditions of the fingers. This might also explain the different results obtained by the application of the 1-MCP on fingers on hands under normal atmosphere and controlled atmosphere. The bananas in the air tight chambers were moister than those exposed to normal atmosphere and consequently their shelf lives were relatively longer at the same concentration of the 1-MCP. However, the moist conditions could not explain the cases where the concentration of the 1-MCP as high as 200 ppm did not show any effect on fingers on the hands while the concentration of 1 ppm significantly did.

In the cases where the higher concentrations (200 ppm) significantly decreased the shelf life (more than 60% of the observation with detached fingers), it was more likely that 1-MCP actively participated in enhancing the rate of ripening. Possibly because it fits at the ethylene binding sites of the fruits, at that much higher concentrations with the fruit losing moisture as it dried up, it was able to trigger off or accelerate similar activities as ethylene usually did.

In the case the concentration of 200 ppm of 1-MCP did not cause any significant difference in the rate of ripening of the banana fingers on hands while it enhanced the rate on the detached fingers it is most likely that the same effect on the detached fingers was not significantly exhibited due to dilution as a consequence of the fingers on hand having more moisture.

Generally, it could be concluded that the effectiveness of 1-MCP in prolonging the shelf life of the bananas under controlled atmosphere at both higher and lower concentrations indicated that the expected low oxygen to carbon dioxide ratio together with damp atmospheres developed inside the chambers, enhanced the prolongation of the shelf life by the very low concentration of 1-MCP, while any prolongation of shelf life at the concentration of 1-MCP of 200 ppm was mainly due to the expected low oxygen to carbon dioxide ratio.

Pelayo *et al.* (2002), concluded in a similar work where 1-MCP was used in collaboration with modified atmosphere, that The potential use of 1-MCP as a supplement to modified atmospheres (at 14 8C) to delay ripening of partially ripe bananas (stages 3 and 4 of ripeness) merits further investigation. However, Zhou *et al.* (2004) reported in their study that 1-MCP together with low temperature storage (modified atmosphere) enhanced the delay in ripening.

In addition, Jiang *et al.* (1999) conclusively reported extension of the shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags (modified atmosphere). In their study, they reported that banana fruit ripening was delayed when exposed to 0.01-1.0 mu l 1-MCP/l for 24 h while longer delays in ripening were achieved with fruit sealed in polyethylene bags (0.03 mm thick) containing 1-MCP at various concentrations.

In this case, as long as the availability of the 1-MCP is not a problem, this method of reducing spoilage of fruits (by using 1-MCP to increase the shelf life) could prove to be inexpensive and easy to put into practical application in regions such as Morogoro in Tanzania as it doesn't require electric power and can involve locally available equipments and material. Thus, farmers, banana wholesalers and retailers would have a significant economic relief albeit the extension in shelf life is about threes days.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

The research came up with results of an over all average of three days shelf life increment by using 1-MCP at the concentration of 1 ppm when the application was done up to the 3rd stage of ripeness. However, the concentration of 200 ppm did not provide an overall average shelf life difference of any significance; on the contrary, it shortened the shelf life of bananas in at least 50% of the treatments.

Of particular importance was the 1 ppm concentration of 1-MCP; this concentration proved to be effective in increasing the shelf life while was more likely low enough to be safe for application on food products. The trend obtained from the results of treatments in this research predicted that concentrations even lower than 1 ppm would be adequately effective in slowing down ripening. This gives 1-MCP more chance for use in the food industry.

In addition to the concentration factor, it appeared that in order to boost the effectiveness of the 1-MCP in retarding ripening, the 1-MCP has to be used under specific optimum conditions with regard to the environment around such as controlled atmospheric conditions. However, it's overall average effectiveness of being able to retard the ripening of banana independently of other factors obtained from this study should serve the purpose of significantly reducing spoilage of banana and improve the conditions of banana retailing as well as general banana production and consumption.

Great caution on the choice of the types of banana might be needed when using 1-MCP for the purpose of shelf life prolongation. This is because it is very likely that it may give different results with different species or variety of bananas, as it had been observed to have variable effects on different types of fruits as described in other researches cited in other sections of this report.

For the case with the retailers as learned from the survey, a three more days longer shelf life would significantly reduce the amount of spoil and the subsequent loss. However the cost attached to the use of 1-MCP with the banana retailing business could not be compared to determine its cost effectiveness.

In this study, some tests could not be performed due to lack of the respective equipments. The tests included measuring the amount of volatile aroma compound, determination of the banana skin colour objectively by using sophisticated equipments, the verification of the predetermined concentrations of 1-MCP by taking direct measurement soon after application, and the determination of the concentration of the carbon dioxide for the case of controlled atmosphere and the ethylene present in general. However, the tests performed yielded results that were practical enough for real life situations with regard to the shelf life of the bananas.

Besides, at this point the availability of 1-MCP would be problematic as the chemical is not yet widely available in various parts of the world, but yet, in time, its availability might become easier and less costly. So, more researches such as this with better equipments would be important in the harvesting of the fruits of science and technology that led to the production of chemicals such as the 1-MCP.

This research could also serve as an initial step of a journey towards hunger alleviation and increased food and nutrition security by prevention of the agricultural loss due to spoilage. One of the future possible beneficial ways of the use of this preservation method would be to enable large scale storage of fruits and vegetables and facilitate long distance transportation of the same to the market, thus contributing to income generation and consequently to poverty alleviation.

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APPENDICES

Appendix 1: Main effects of the 1-MCP, storage conditions, nature of fingers, and their interaction

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1300.792(a)	11	118.254	32.319	.000	.552
Intercept	18411.341	1	18411.341	5031.782	.000	.946
Fingers	397.826	1	397.826	108.725	.000	.274
MCPconc	417.504	2	208.752	57.051	.000	.284
storage	264.588	1	264.588	72.311	.000	.201
Fingers * MCPconc	192.028	2	96.014	26.240	.000	.154
Fingers * storage	13.372	1	13.372	3.655	.057	.013
MCPconc * storage	97.644	2	48.822	13.343	.000	.085
Fingers * MCPconc * storage	59.542	2	29.771	8.136	.000	.053
Error	1053.795	288	3.659			
Total	22658.000	300				
Corrected Total	2354.587	299				

a R Squared = .552 (Adjusted R Squared = .535)

Appendix 2: The effect of 1-MCP concentration, storage conditions, and state of fingers on mean shelf life of ripe bananas

Fingers	Concentration of 1-MCP in ppm	Storage conditions	Mean shelf life (days)
Fingers detached	0	Normal atmosphere	8.6± 3.8
		Controlled atmosphere	8.5± 2.2
		Total	8.5± 3.2
	1	Normal atmosphere	10.4± 3.1
		Controlled atmosphere	16.1± 1.0
		Total	13.3± 3.7
	200	Normal atmosphere	7.0± 0.0
		Controlled atmosphere	9.0± 3.1
		Total	8.5± 2.8
	Total	Normal atmosphere	8.6± 3.3
		Controlled atmosphere	10.2± 3.9
		Total	9.6± 3.7
Fingers on hand	0	Normal atmosphere	6.0± 0.0
		Controlled atmosphere	7.5± 0.5
		Total	6.7± 0.8
	1	Normal atmosphere	7.0± 0.0
		Controlled atmosphere	9.2± 1.2
		Total	8.3± 1.4
	200	Normal atmosphere	6.8± 1.0
		Controlled atmosphere	7.9± 0.8
		Total	7.3± 1.1
	Total	Normal atmosphere	6.5± 0.8
		Controlled atmosphere	8.1± 1.1
		Total	7.3± 1.2
Total	0	Normal atmosphere	7.2± 2.9
		Controlled atmosphere	8.0± 1.6
		Total	7.5± 2.4
	1	Normal atmosphere	8.9± 2.8
		Controlled atmosphere	12.2± 3.7
		Total	10.7± 4.0
	200	Normal atmosphere	6.8± 0.9
		Controlled atmosphere	8.4± 2.3
		Total	7.7± 2.0
	Total	Normal atmosphere	7.3± 2.3
		Controlled atmosphere	9.1± 3.0
		Total	8.2± 2.8