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**ACTIVE PACKAGING IN MASTER BAG SOLUTIONS
AND SHELF LIFE EXTENSION OF
RED RASPBERRIES AND STRAWBERRIES:
A RELIABLE STRATEGY TO REDUCE FOOD LOSS**

Scientific Field AGR\15

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ABSTRACT

A rough estimation for avoidable losses in the European Union (EU) is 280 kg per capita per year, of which 13% can arise from agricultural production, 31% from product processing and 45% from households. The role of packaging in preserving fresh and processed foods is well known and documented but little research is available about the relation existing among new packaging solutions, shelf life extension and Food Loss, and waste reduction at different levels at the supply chain. Techniques as Life Cycle Assessment (LCA) have been largely used to determine the Environmental Impact of food production and processing and packaging materials. However, the assessment taking into account the food and its packaging as a whole system, and of Food Loss reduction is necessary. In fact, from a life cycle perspective, no assessment of the Environmental Impact of food packaging showed the positive benefits of reduced Food Losses in the value chain.

In this PhD project, the shelf life extension of red raspberries (*Rubus idaeus* L.) and strawberries (*Fragaria x Ananassa* Duch) using active packaging solutions was investigated. The shelf life extension, critical indicators and cut-off criteria were defined as a tool to point out the time at which the lifetime ended and they were elaborated by multivariate approach. The final aim was to estimate the role of a new packaging technology in reducing the Environmental Impact along the supply chain in relation to the benefits of the Food Loss reduction derived from the actual Shelf Life Extension.

For raspberries trial, three packaging solutions was studied: a) Lidded macro-perforated PET trays containing 125 g of berries, stored in air and considered as “traditional” packaging; b) lidded macro-perforated PET trays containing 125 g of berries inserted into master bags made of plastic materials with different permeabilities to gas and water vapour. This solution was referred to a passive modified packaging solution. c) macro-perforated PET trays containing 125 g of berries inserted into a master bag unit made of LDPE (OTR 4000 cm³*m²*day⁻¹ at 23 °C and 0 %RH). Before sealing, a defined volume of compressed dry air (moisturized by using distilled water applied onto paper towels), one oxygen scavenger, and a different number of pre-activated carbon dioxide emitters were added to the master bag.

For strawberries trial three packaging solutions was studied: a) Lidded PET macro-perforated trays containing 250 g of fruits and stored in air were considered as “traditional” packaging; b) lidded PET macro-perforated trays (250 g of berries/tray) were inserted into an LDPE (OTR 4000 cm³*m²*day⁻¹ at 23 °C and 0 %RH) master bag. c) A different number of PET macro-perforated trays were inserted into an LDPE master bag. A central composite design (CCD) with four factors (number of CO₂ emitters, number of O₂ scavengers, ratio between packaging surface area and unfilled volume, storage time) at five levels was performed to optimize the active packaging solution.

All the samples were stored in a cold chamber (5±1 °C; 70±5 %RH).

Different physical-chemical and sensorial analyses were performed as following to identify for each packaging solution the shelf life value: Damaged berries (%), Mouldy berries (%); Weight loss (%); Colour (CIE L*, a* and b* parameters); Total solids (g/100g); Soluble solids (g/100g); pH; Titratable acidity (g citric acid/100 g); Consistency determined by single compression test (force*deformation at 60% of deformation); Volatile compounds by SPME-GC-MS technique; Sensorial global and Visual acceptability. In order to analyze the results from a multidimensional point of view, the obtained data were analyzed by Principal Component Analysis (PCA).

Il ciclo di vita per ogni soluzione di imballaggio è stata valutata utilizzando il software 8.0.1 SimaPro®. The boundaries of the system was set from the berries production until the retailer storage, take into consideration also the operations to disposal of the packaging materials. The functional unit for this study was set as the day of shelf life.

In the raspberries studies, the active packaging solution allowed the raspberries storage up to 11 days. This value was almost three times longer than the “traditional” packaging solution that allowed a shelf life value of 4 days. The Passive packaging solution allowed lead to a shelf life extension as 2 days in comparison to the traditional packaging.

For each packaging solution have been done the assessment of the environmental impact using the LCA methodology.

The “traditional” packaging solution determined the highest daily impact among the packaging solutions evaluated. The passive and active packaging solutions determined a significative reduction, in terms of environmental load, up to 55% and 70%, respectively. The extension of the lifetime of berries contribute also to reduce the food loss even if the environmental impact of packaging system (active devices and master bag) was increased. This increment was balanced from the environmental impact of food saved by using the new packaging solution.

The assessment of strawberries shelf life stored in the traditional packaging systems established only 2 days as value, while in the passive packaging solution lead to a shelf life extension up to 4 days longer. The optimization of the packaging factors in the active packaging solution extended the berries storage until 12 days.

The “traditional” packaging determined the highest daily impact than the other packaging solutions. The passive and active packaging solutions determined a significative reduction in environmental load respect to the “traditional” solution up to 66% and 82%, respectively.

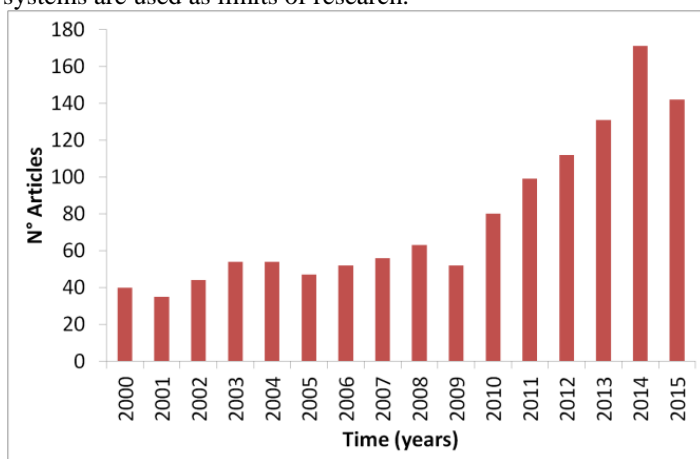
The implementation of LCA methodology with lifetime data assessed by experimental shelf life trials and multivariate analyses allowed the definition of the impact of new technologies based on active packaging, taking into account their role in shelf life extension. Although based on some assumptions, this PhD study tried to explain and measure how new packaging can affect fruits losses directly or indirectly by influencing the scenarios at different levels of the supply chain. The lack of economic and logistic information about Food Losses (and specific data on this kind of fruits) in the Italian supply and distribution chain should open to new and more useful considerations.

1-INTRODUCTION

Preface

The issue of overall Food Losses and Waste (FLW) has lately received much consideration and it was given high visibility. In fact the number of papers dedicated to this topic has increased considerably in recent years (Figure 1.1).

Figure 1.1. Articles written from 2000 to 2015 about the Food Waste and Loss; research conducted in SciFinder using Food Waste and Loss as key words. Environmental, Food waste, and Food systems are used as limits of research.



This preface considers two main aspects:

First, a concern related to food insecurity and hunger. The Food and Agriculture Organization estimates that about 795 million people of the 7.3 billion people in the world suffer from chronic undernourishment in 2012-2014 (FAO, 2014). This situation seems to indicate that something is wrong in the food system, the efficiency of the food chain have chronic problems, although no one has yet established the direct link between the issue of FLW and the food insecurity. The reduction of FLW is now presented as essential to improve food security (HLPE, 2011; FAO, 2012a,b). However, real causes of hunger and malnutrition are very complex and cannot be reduced to the existence of FLW.

Second, a concern related to the impact of FLW on environment, i.e. the capacity of ecosystems and natural resources to sustain an increasing demand for food, driven by population and income growth and changing consumption patterns (FAO, 2012a). In this perspective, FLW represents at the same time a loss of natural resources and an environmental impact described as Ecological-Carbon and Water footprint.

The consequences of FLW have an important effect not only on the environmental but also on the social and economic perspectives. FLW tend to become a symbol of the inefficiency, unfairness and unsustainability of food systems. Their reduction seems a priority to improve the sustainability of food systems (HLPE 2014).

The proposal of the United Nations is the zero-food loss and waste challenge to reach the 100% sustainable food systems. *“The key to better nutrition, and ultimately to ensuring each person’s right to food, lies in better food systems – smarter approaches, policies and investments encompassing the environment, people, institutions and processes by which agricultural products*

are produced, processed and brought to consumers in a sustainable manner”, Secretary General Ban Ki-Moon said in his message for the World Food Day on 16 October 2013 (UN, 2013).

In the recent past the most significant efforts in food science and technology have been addressed to extend the commercial life of foods and beverages. In this context, however, very little or null attention has been paid to the possible positive contribution, coming from a shelf life extension (SLE) to the overall sustainability of a food product along its entire supply chain. Nevertheless, a shelf life extension can contrast food losses and the distribution logistic impacts and several studies stressed the importance of increasing the knowledge about these issues.

The overall aim of this PhD project is to match a Shelf Life Extension (SLE), due to a packaging innovation, to the possible increase of global sustainability of a food system along the supply chain in terms of environmental load and food loss reductions. It means to learn how to use the SLE as an Indicator of Sustainability, i.e. to demonstrate, when possible and feasible, that a longer shelf life means also higher sustainability (PRIN, Piergiovanni 2014). Red small fruits (raspberries and strawberries) will be presented as case studies as shelf life extension due to packaging technologies has not been yet investigated and food losses along the supply chain still represent an important issue in the sustainability .

1.1 FOOD LOSSES-WASTE AND SUSTAINABLE FOOD SYSTEMS

1.1.1 Definition of FLW

The terms of Food Waste and Food Loss have been defined in different ways in literature it is common to find a distinction between these two terms (FAO, 2011a; Parfitt, Barthel and Macnaughton, 2010) but in some cases the distinction is not so clear and unambiguous.

A first definition is based on the distinction between Food Loss and Food Waste taking into account the step of the food chain at which the loss or waste of food physically happens (HPLC 2014); food loss happens along the food supply chain, and food waste happens at the end of the supply chain, towards the consumer step.

A second definition take into account the source and the cause of loss or waste. In particular, some Authors tried to link it to the nature or origin of the cause of loss or waste, considering whether its cause is “behavioural” (waste) or not (loss); “voluntary” (waste) or not (loss); the result of an explicit choice (waste) or not (HPLC 2014).

This approach amplifies the issue giving a different and often subjective attitude towards what these terms mean in different contexts.

A third definition has been coined by some authors that use “food waste” or “food wastage” as a generic mean for “food losses and waste”, which has the limitation that often some of this “waste” is in fact, under other approaches, a “loss”. This gets further confusing when authors expand the scope to all “food-related” waste, which includes non-edible parts (WRAP, 2008)

In this thesis, definitions ascribed to first group will be used as follows:

Food losses (FL) refers to a decrease, at all stages of the food chain prior to the consumer level, in mass, of food that was originally intended for human consumption, regardless of the cause.

Food waste (FW) refers to food appropriate for human consumption being discarded or left to spoil at consumer level, regardless of the cause.

In this last definition the main distinction between FAO and Waste and Resources Action Program (WRAP, 2009) focuses the attention on the “Avoidable” and “Unavoidable” food waste, where in the first case (Avoidable) the food that is thrown away still being edible (for example, slices of

bread, apples meat, etc.), while the Unavoidable food waste concerns the waste deriving from the preparation of food or drinks (for example, meat bones, egg shells, pineapple skin, etc.). Nevertheless, it is necessary to highlight that in the estimation of the food waste the Unavoidable food is not taken into account in the FAO approach while in the WRAP reports it is. This diatribe could lead to a divergence problem in those works that try to evaluate food waste.

1.2 THE DATA OF THE ISSUE OF FLW

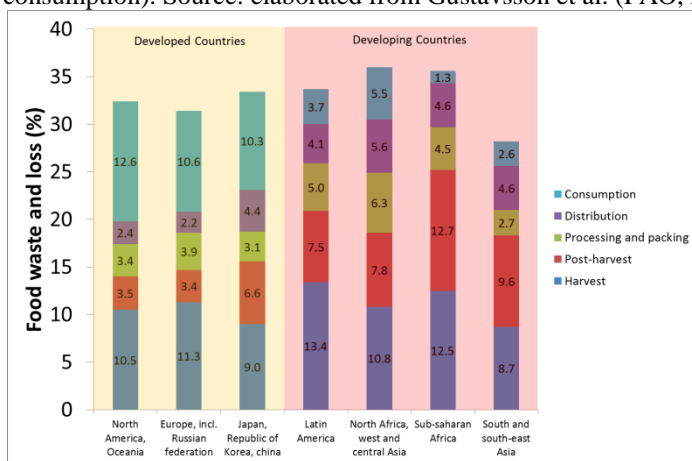
Data about FLW are reported in different reports and, as mentioned above, it is difficult to match data coming from different studies. For this reason some discrepancies among the data presented below could occur.

1.2.1 The global scenario

One of the few global analyses available is a study carried out in 2011 by FAO, which estimated an annual global waste at approximately 1.3 billion of tons that correspond roughly to one third of the whole world food production.

The distribution of food waste and loss depends on the country and the phase of supply chain. Gustavsson et al. (FAO, 2011) defined an estimation of FLW for different world macro-area and for steps of supply chain as shown in Figure 1.2.

Figure 1.2. The bars represent the percentages of food lost or wasted at each step of the chain, expressed in percentage of the initial production (edible part originally intended for human consumption). Source: elaborated from Gustavsson et al. (FAO, 2011a).



The distinction between the two macro-areas can be summarized in the shift of the FLW in different stages along the supply chain. The main step where the FLW happens is during the harvest but at this stage it is difficult to reduce the amount because, in most of cases, it depends on the climatic conditions. After this step, the developing countries lost and wasted the food during the post-harvest step due to the lack of facilities (e.g. cold chambers and refrigerated trucks), infrastructure (e.g. highway and railway), and knowledge of the food storage (e.g. using the modified atmosphere). In the developed countries the issues that occur in the developing countries has been resolved; FLW in the developed countries are located in the consumption step due to the abundance of food in household and the incorrect respect on the value of food that lead to spoil the food in the fridge or in the pantry.

1.2.2 Europe scenario

In Europe, the Eurostat (2010) estimates that the quantity of food wasted in Europe every year amounts to 89 million tons, or 180 kg per capita. The average came from a large range of food waste amount starting from Greece with 44 kg per capita per year, passing through Italy with 149 kg per capita per year and ending with Netherlands with 579 kg per capita per year).

In table 1 the percentage of FLW during the different steps is represented. For example, in the fruits and vegetables category, the FLW is more than 50 % taking into account the overall system (Table 1.1). This estimation is in agreement with other studies that report the same results for this category (WRAP 2008 and BCFN 2012).

Table 1.1 Estimated/assumed waste percentages for each commodity group in each step of the FSC for Europe including Russia. Source: from Gustavsson et al. (FAO, 2011a).

Foods	Steps				
	Agricultural production	Postharvest handling and storage	Processing and packaging	Distribution: supermarket retail	Consumption
Cereals	2%	4%	10%	2%	25%
Roots and tubers	20%	9%	15%	7%	17%
Oilseeds and pulses	10%	1%	5%	1%	4%
Fruits and vegetables	20%	5%	2%	10%	19%
Meat	3.1%	0.7%	5%	4%	11%
Fish and seafood	9.4%	0.5%	6%	9%	11%
Milk	3.5%	0.5%	1.2%	0.5%	7%

As mentioned above, the food waste and loss determine economic and social impacts but in recently years the attention was shifted also to environmental load.

Recent studies have estimated the amount of natural resources used when food is lost or wasted. Most of these works uses a simple proportional estimation of the environmental load of food production, applying the same average value to the amount of food estimated to be lost, without taking into account the step where the FLW happened (HLPE 2014). Unfortunately this correlation denotes a very roughly estimation due to the approximation of the data.

Life cycle analysis methodologies can be used to estimate better the environmental load of FLW taking into account the step where this issue happens and the “end of life” of the system. In common and advertisement perspective this study can be called “footprints” that measure the various ways resources used or needed, or external impacts generated throughout the life cycle leading to the production and discard of food.

The carbon footprint of global FLW, without accounting for Greenhouse Gases (GHG) emissions from land-use change, is estimated to be 3.3 Gtonnes of CO₂ equivalent, an amount equivalent to 6–10 percent of anthropogenic greenhouse gas emissions (Vermeulen et al., 2012). In Italy, in 2009, the fruits and vegetables lost during the distribution step produced 8.4 million of kg of CO₂ (Segrè and Falasconi, 2011).

Food loss and waste include also water “waste” (Lundqvist, de Fraiture and Molden, 2008), as large quantities of water are used to produce the food. From the environmental perspective, food losses and waste account for more than one-quarter of the total consumptive use of finite and vulnerable fresh water and more than 300 million barrels of oil per year (HLPE, 2014). In Italy, in the distribution step, the fruits and vegetables lost have used to grow 73.8 million of m³ of water (Segrè and Falasconi, 2011).

According to FAO (2013a), the surface used to grow the crops and the animals, that became FLW, occupied about 1.4 billion hectares of land; this represents close to 30 percent of the world's agricultural land area. In a study on global resource productivity practices by the McKinsey Global Institute (Dobbs et al., 2011), reducing food loss and waste was ranked in the top three measures that will contribute to improve productivity of resources, pointing to the fact that a reduction of consumer food waste in developed countries by 30 percent would save roughly 40 million hectares of cropland. In Italy the fruits and vegetables lost during distribution step have occupied 390 million of m² of global land (Segrè and Falasconi, 2011).

Finally, in terms of environmental impacts, it is important to note that consumer food waste has a greater carbon, GHG, land-use, water, nitrogen or energy footprint than a similar mass of post-harvest loss. This is due to the inclusion of the footprints of transport, packaging, processing, distribution and preparation at home, all of which is finally “embedded” in consumer waste. For instance, an average consumer waste is equivalent to eight times more energy “waste” than post-harvest loss (Dobbs et al., 2011).

The performance of food systems can be increased in term of economic, social and environmental effects by increasing the efficiency of the systems. Therefore FLW is an important issue when considering the challenge of feeding the world in 2050 (Bruinsma, 2009), as well as challenge of ensuring food security in a context of climate change (HLPE, 2012). The estimation of efficiency improvements in the food system is a key point for the evolution towards sustainability.

1.3 CAUSES AND DRIVERS OF FOOD LOSSES AND WASTE

The knowledge of the causes of food loss and waste is the first step to plan the actions to decrease this issue.

The causes of FLW were located in different steps along the supply chain from harvest to consumer and, in a macro scale, in the management of food system and chain, and also in the social, economic and environmental policies and international cooperation.

The following paragraphs will explain the causes and drivers of FLW for each step along the supply chain of fruits and vegetables:

PRE-HARVEST: the factors that drive the FLW can be divided into four categories: 1) the choice of crop species and cultivars taking into account the pedoclimatic conditions can contribute to reduce the food loss due to the resistance of variety against environmental condition and plant disease/pest, meeting the target of market requirements; 2) good management of agronomic activity like fertilization, pest treatment and irrigation, can contribute to increase the quality and quantity of production preventing also the metabolic disorder and resistance against environmental and plant disease/pest; 3) biological factors such as diseases or nutritional deficiency can produce or reduce the FLW; 4) environmental factors, i.e. too wet or too dry periods can develop physiological disorder or growth of disease. Biological (internal) causes of deterioration include respiration rate, ethylene production and action, rates of compositional changes (associated with color, texture, flavor, and nutritive value), mechanical injuries, water stress, sprouting and rooting, physiological disorders, and pathological breakdown. The rate of biological deterioration depends on several environmental (external) factors, including temperature, relative humidity, air velocity, and atmospheric composition (concentrations of oxygen, carbon dioxide, and ethylene), and sanitation procedures

Sometimes failure to harvest is due to the economic reasons such as low market price at the time of harvest and high labour cost. If the crop matures when the demand is low some producers opt to leave the crop in the field as the returns do not justify the cost of harvesting and transport. In Italy in 2009, 17.7 million tonnes of agricultural produce were left in the fields, representing 3.25% of total agricultural production (Segrè and Falasconi, 2011).

HARVEST: The period of harvest is decided not only by nature (biologic and weather) but also by market requirements and economic factors. Often these elements do not find the correct point of interaction to reach the reduction of the food waste and loss. The perishable products like fruits and vegetables have to be harvested in a short period but in the case of a too wet weather, inadequate product quality, or when there is no market demand for these products, the vegetables and fruits can be left of the field; in same case to avoid this problem the products are collected before the mature but the quality is lower than requirements (Kader 2005). In other case the technique of harvest can contribute to the loss because the market requirements are too strict.

STORAGE: In developed countries the storage facilities are well organized from harvest to retailer. Cold storage with post-harvest technologies can be able to extend the shelf life and marketing period especially for perishable foods. In this condition the FLW occur due to a breakdown of refrigeration systems or temperature abuse.

In developing countries the main cause for FLW is the lack of storage facilities (FAO 2011). Without that equipment (e.g. cold chamber to control the correct temperature and humidity and gas composition) the perishable products can be thrown away due to the acceleration of degradation or the growth of hazardous microorganisms and substances.

TRANSPORT-LOGISTIC: In developed countries, as above described, the facilities of logistic are well organized and coordinate. In this condition only trouble or breakdown during transport or coordination can cause loss.

In developing countries lack of proper transportation vehicles, poor infrastructure and inefficient logistical management make the improper condition for product conservation (Roll 2006). The difficult weather conditions during particular periods of the year can stop the transportation of food product which gets spoiled. It is estimated that post-harvest losses of fruits and vegetables can range from 35 to 50 percent annually due to poor infrastructure (IMechE, 2013).

PROCESSING AND PACKAGING: Loss of proper process management or inadequate knowledge or inefficient structures to process the product can cause unsafe and nutritionally poor products. For example an improper blanching of fruits or vegetables doesn't arrest the enzymatic activity and causes off-flavour and discoloration of the processed product, which may be discarded.

Packaging can be an important element to extend shelf life and prevent FLW (FAO 2011) in particular due to the adequate design or technologies used to made it (Williams et al 2012).

RETAIL: The facilities and technologies used during retail step can contribute to extend or decrease the shelf life of product; in fact using cold display or not can alter the conservation of fruits and vegetables. Using MAP solution in the packaging system can maintain the product at good quality for a longer period.

In the USA, it was estimated that the in-store food losses were 10% of the total food supply (Buzby 2014).

The tendency to propose homogenous and perfect product (in terms of colour, shape, size, freedom from blemishes) have led most retailers to set high standards for products. The failure in fitting these standards determines a rejection at delivery or a negative consumer judgement. Moreover, these strict conditions impose a high grading by the growers causing food loss.

CONSUMPTION: The consumption step is one of the most important phases in food waste especially in the developed countries. As shown above the numbers related to this problems are important but the causes can be gathered in few points collected (WRAP 2009; HISPACOOOP 2012; Baptista et al. 2012).

- no planning or incorrect planning of purchases often leading to buy more than needed (impulsive or advance purchasing of food that is not required immediately improved by the advertising at retailer such as "economic packaging" or "discount" or "three-for-two");
- discarding food due to confusion over "the best-before" and used-by" dates (Table 1.2);

- improper food storage or stock management at home, e.g. keeping the fruit out of the fridge during the warm-hot seasons;
- excess portions prepared and not eaten;
- inadequate wrapping and use of materials, which affect a healthy preservation of food and reduce the consumption period, e.g. changing the original proper packaging material with another considered more “healthy”;
- poor food preparation techniques often leading to less food being eaten, to food quality losses and to waste due to the preparation method; lack of knowledge on how to consume/use food more efficiently, e.g. use of the leftovers inspired by different recipes instead of discarding.
- lack of awareness of the amount of waste one produces and its economic and environmental impact.

Table 1.2. Correct meaning of “Best Before” and “Used-by Date” Source: HLPE, 2014

<p>“Date of Minimum Durability” (“Best Before”)</p>	<p>the date that signifies the end of the period under any stated storage conditions during which the product will remain fully marketable and will retain any specific qualities for which tacit or express claims have been made. However, beyond the date the food may still be perfectly satisfactory</p>
<p>“Use-by Date” (Recommended Last Consumption Date, Expiration Date)</p>	<p>the date that signifies the end of the estimated period under any stated storage conditions, after which the product probably will not have the quality attributes normally expected by the consumers. After this date, the food should not be regarded as marketable.</p>

Moreover, other aspects in meso-macro scale exist that have an effect on the production of FLW. One of this is the **improper definition of the shelf life** because the potential inaccuracies in its definition may be responsible of two different scenarios: a shelf-life overestimation that could cause consumer complaints, product recalls, ineffective logistic impacts, food losses, etc... and a shelf-life underestimation that could cause serious, expensive food losses and wastes.

All foods are susceptible to quality and safety losses. The shelf-life can be defined as a finite length of time after production and packaging during which the food product retains a required level of quality under well-defined storage conditions; therefore, shelf life should reflect only the quality loss dynamics (Nicoli, 2012). The definition of shelf life requires a multidisciplinary approach because different driving forces are involved: regulatory, economics, marketing, social. Since the pioneer work from Labuza and co-workers (Labuza, 1982) many articles in literature focused the attention on the shelf life of packaged food products; unfortunately, few papers only assessed the shelf life in a correct way determining the date where product was failing.

The first step in assessing the shelf life is to identify the main parameter that describes the food quality decay of packaged food during storage time. It is possible to use traditional techniques (e.g. chemical analyses, physical analyses or sensory attributes) or to exploit otherwise an innovative procedure gathering different decay-parameters as multivariate analysis (Pedro and Ferreira; 2006) or assessing the maximum rate of degradation reactions (Limbo et al., 2009).

The second step is to set the acceptability limit determining the value (or the range of values) that describes whether one product is acceptable or not from the consumers. Usually industry and researchers do not specify this depletion index in a measurable way; its determination is complicated and in many papers this index is not evaluated or is arbitrary set. In agree with the recent book of Nicoli (2012) many ways to determine the acceptability are available; regulation

parameters and consumer satisfaction with sensory feature or nutritional aspect determined by instrumental analysis can be used. In the last years, the role of food-consumer interaction has progressively increased, driving the shelf life approach from the food product to the consumer's judgments (Gacula, 1975).

The last step in shelf-life assessment is to monitor the critical indicator under real-time or accelerated conditions of storage to evaluate quality changes. Data collected during testing are modeled to obtain parameters able to describe the kinetics behaviors, thus to predict the shelf life once the acceptability limit has been defined. These models should take into account the effects of environmental factors like gas pressure, moisture, light intensity temperature, gas permeability of packaging materials, etc... that can interfere with the food quality over the storage. Therefore few strategies have been outlined, especially when more than one accelerating variable or when accelerating factors different from temperature are used to speed up the quality decay during experiment.

From this point of view there is a need of defining a protocol to identify the correct shelf life and to develop a sustainability-trend.

Techniques such as Life Cycle Assessment (LCA) can be used to quantify environmental impact of the food losses and waste arising from an incorrect definition of shelf life. This approach is in accord with a recent study (Wikström and Williams, 2010) which stressed the importance of increasing the knowledge about the amount of food losses, the environmental impact due to losses and the reasons why losses arise. In fact about 15-25% of the climate impact of consumption is caused by food and nutrition (Seppälä et al.,2009).

Finally, knowing quite well the shelf life of a packaged product, it might be possible to re-design the package or the distribution logistics, in order to save time and reduce environmental burden. After the evaluation of the food waste causes, the solutions are easier to be identified but often difficult to be applied. At each step of the supply chain the causes of FLW are reduced applying the good practices of production, managing the resources, and improving the whole food system also in terms of facilities and infrastructures There is not only one way to present this problem and correct bad-manners of consumers. Many organizations and associations explain the problem at each level, trying to educate the people, children and adults, to reduce the food waste and improve the good-manner to reach a sustainable food system.

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2- AIM OF THE THESIS

A rough estimation for avoidable losses in the European Union (EU) is 280 kg per capita per year, of which 13% can arise from agricultural production, 31% from product processing and 45% from households. The role of packaging in preserving fresh and processed foods is well documented, but little is known about the relation existing among new packaging solutions, shelf life extension and waste reduction at different levels at the supply chain.

The PhD project was firstly focused on the shelf life assessment of strawberries (*Fragaria x Ananassa* Duch) and raspberries (*Rubus idaeus* L.) using new packaging solutions based on master bag and active packaging in order to extend the shelf life of these fruits. Critical indicators and cut-off criteria were defined and they were elaborated by multivariate approach in order to point out the time at which the lifetime ended allowing the set up the shelf-life extension.

Secondly, the Shelf Life Extension (SLE) obtained through packaging innovation, was matched with the possible increase of global sustainability of a food system along the supply chain in terms of environmental load and food loss reductions for both strawberries and raspberries. The SLE can be considered an Indicator of Sustainability since the evidence that a longer shelf life means also higher sustainability, when possible and feasible, exists. Moreover, the Environmental Impacts of the new packaging solutions adopted were calculated by means of the Life Cycle Assessment. The benefits of the Food Loss reduction derived from the actual Shelf Life Extension can be estimated.

3 - SHELF LIFE ESTIMATION OF RASPBERRIES PACKAGED IN “TRADITIONAL” PET TRAY

3.1 INTRODUCTION

As defined above, the shelf life is the time at which the product is not acceptable from consumer or it is not safe for consumption and the concept of shelf life estimation can have effect on the production of food waste and loss. In this PhD thesis section, a protocol to determine the shelf life of raspberries packaged in “traditional” solution is presented. In fact the improper definition of shelf life may be responsible of two different scenarios: a shelf life overestimation that could cause consumer complaints, product recalls, ineffective logistic impacts, food losses, etc and a shelf life underestimation that could cause serious, expensive food losses and wastes.

Many reviews and books have been written to describe the methodologies applied for the definition of the shelf life value but each author use a specific grouping for the possible methodologies (Nicoli 2012; Hough 2010; Robertson 2009). The most used methodology starts with the determination of the critical indicators, evaluating the experimental data (which is the main factor that represents the quality decay) or consumer satisfaction or historical market data (especially in the industrial field). To define the time at which the critical indicator reaches its limit it is necessary to define the critical limit (acceptability limit). In this contest, it is possible to follow different strategies. The first is the use of an arbitrary value, defined by the authors or the company. It is clear that this approach is not so efficient to determine the shelf life (Portela and Cantwell, 2001). The second approach is based on sensorial tests performed by trained panels or by consumers to determine a sensorial value at which the samples are still acceptable; this procedure can be expensive and does not necessarily represent the rejection of product (Gambaro et al., 2006). A third approach is based on the failure criteria in survival analysis where the shelf life is a time limit at which a given percentage of food products is expected to fail and the acceptability limit is then chosen by selecting the percentage of food failure considered tolerable (Hough 2009). Another approach is based on a legislative limit, for example the maximum concentration of microorganism accepted to grow on the surface of product or the respect of the values presented on the label, for example the value of vitamin C in fruit juice (Polydera et al., 2005). In some cases it is possible to determine the limit using the time at which the kinetic of degradation of the critical indicator has the maximum acceleration or the maximum rate (Limbo et al., 2009).

After the definition of the acceptability limit it is necessary to model the critical indicator and set the limit on the model to define the shelf life value. This procedure can be adopted in the real condition but it can be applied also on accelerated storage conditions.

For these reasons it is not easy to define with high accuracy the shelf life value, especially in those products where the expired date is not expressed (see the whole fruits).

For example, in this case the problem becomes even more complex because numerous factors have to be studied together and for each one of they, the limit have to be defined. Especially in the sensory analysis, is not easy define which is the most appropriate to estimate the shelf life value (Pedro and Ferreira, 2006). Multivariate techniques of analysis present a set of useful tools for shelf-life studies in which several properties need to be monitored. One of the most applied techniques is Principal Component Analysis (PCA), which aims at finding a new set of axes in multivariate space that better describe the structure in the data. These new axes are called Principal Components (PC) and are built by linear combinations of the original variables (Malinowski 1991; Wold et al., 1987).

In this part of the work, two different approaches to determine the shelf life value of raspberries packed in the traditional system (defined as the macro-perforated PET tray with macro-perforated PET lid) were compared. The objectives of this work were, also, the definition of the critical quality index (or indices) and its limit (or their limits). The small red fruits and in particular

raspberries (*Rubus idaeus* L.) have very short storage life due to the physiological aspects such as high respiration rate, loss of firmness, mould susceptibility and breaking down tissues.

3.2 MATERIALS

Red Raspberries (*Rubus idaeus* L.) cv. Erika from Northern Italy picked at a commercial ripening state were provided by a local supermarket in Milan and transported to the laboratory where they were immediately stored in a dark cold chamber (5 ± 1 °C, 70 %RH) before packaging. The raspberries were purchased in a macro-perforated PET tray (9.5x14x4.5 cm) with a PET rigid lid: this is the traditional sale unit containing 125 g of fruits.

The changes in quality during storage were monitored in 3 different periods, July and October 2013 and October 2014.

3.3 METHODS

3.3.1 Sensory Shelf life

Survival analysis: Forty consumers, approximately balanced between males and females, were recruited among students and employees from the University of Milan (Italy); they were between 21 and 60 years old and they regularly consumed raspberries. At each sampling time, consumers were asked to visually evaluate the acceptability of raspberries in each packaging condition, responding “yes” or “no” to the following question: “Imagine you are in the supermarket to buy raspberries, would you normally consume this product?”.

The parametric model was used to define the time at which the 50% of consumers rejected the product (Lareo et al., 2009; Gámbaro et al. 2006; Giménez et al. 2007), when stored in the traditional sale unit (macro-perforated PET tray). Then, this limit was applied to identify the instrumental limit of acceptability of the main quality parameter and thus estimate the shelf life. For the survival analysis the software R (Bell Laboratories, University of Auckland, New Zealand) was used.

Consumer acceptability: Forty consumers, approximately balanced between males and females, were recruited among students and employees from the University of Milan (Italy); they were, between 21 and 60 years old and they regularly consumed raspberries, approximately balanced between males and females. At each sampling time, consumers were asked to visually evaluate the acceptability of raspberries in each packaging condition. The consumers were asked to give an overall appreciation of raspberries quality on a 1-9 scale: 1 -extremely bad- to 9 -extremely good- (Ares et al. 2008). In the case of acceptability scores 1–5 the rating was transformed to the word “no”, indicating that the consumer disliked the product. In contrast, if a consumer's score for a sample was 6–9, it was replaced by the word “yes”, indicating that the consumer liked the product (Giménez et al. 2008). The data were used to calculate the percentage of the consumer that appreciated the berries at each storage time.

Visual colour evaluation: The berries colour was also assessed by the same forty consumers using a 1 to 5 visual rating scale (Perkins-Veazie and Nonnecke, 1992).

3.3.2 Chemical and Physical analyses

Unacceptable berries: Both physically damaged and mouldy berries were visually estimated at each sampling time with results expressed as percentage of unacceptable berries (Van deer Steen et al., 2002).

Colour: At each sampling time, superficial colour of the berries (L^* , a^* and b^* parameters) was measured on 30 fruits taken from three different packages using a handheld Tristimulus colorimeter (Konica Minolta CR-300, Tokyo, Japan) with a 8 mm diameter, 2° standard observer and a C as illuminant source. Before each measurement, the apparatus was calibrated on the Hunterlab color space system using a white ceramic tile (Minolta calibration plate, $Y = 92.6$, $x = 0.3136$, $y = 0.3196$). Colour was described as Hue angle (H° , expressed as $\arctg b^*/a^*$) and Chroma (C, expressed as $(a^{*2} + b^{*2})^{1/2}$) indices.

Weight loss: A gravimetrical determination was performed by weighting each PET tray at time zero and during the storage using a Technical balance (MP-3000 Chyo Balance corp., Japan). Changes in fruit weight were expressed as percentage.

Firmness of raspberries: A single compression test on each berry (modified method from Sousa et al., 2007 and Giovanelli et al., 2014) was performed using a dynamometer (Zwick Roell Instrumental Z010, Zwick GmbH & Co. KG, Ulm, Germany). At least 30 berries were assessed per period. Each berry was positioned under the probe plate (80 mm diameter) and compressed to 60 % deformation using a load cell of 10 kg (100 N), at 2 mm/s test speed and with 5 g pre-load. The method used to assess product firmness intended to measure the structure resistance against the compression applied to the top (peak)-bottom direction. The labour was measured at 60 % of deformation, describing the cavity complete collapse, when the deformation overcomes 60 %, the instrument measures the drupelets resistance to compression.

Dry matter content: Determinations were made on 5 g of fruit pulp by drying samples in an oven set at 105°C . The samples were weighed after about 16 hours. The measurements were done in triplicate. The results were expressed as g of dry matter per 100 g of sample.

Titrateable Acidity (TA): After thawing of samples at 4°C overnight, TA was determined by titrating sample (2 g of homogenate + 40 mL of CO_2 -free distilled water) with standardized 0.1 N NaOH to pH 8.2 by use of a pH meter (Basic 20+, Crison Instruments SA, Barcelona, Spain). TA was expressed as citric acid equivalents (grams of citric acid per 100 grams of berries).

Total soluble solids (TSS): After thawing of samples at 4°C overnight, measurements were done on berries pulp using an Automatic Refractometer model SMART-1 Atago®, (Atago CO.LTD, Tokyo, Japan). The results was expressed as BRIX°.

3.3.3 Statistical analysis

Data were statistically evaluated by one-way ANOVA and multiple range test (Tukey method) to put in evidence significant differences among samples, using Statgraphics Plus v. 5.1 package (Statpoint Technologies, Inc. Warrenton, VA 20186, USA). The differences were considered significant at $P < 0.05$. In order to analyse the results from a multidimensional point of view, the quality parameters data were analysed by Principal Component Analysis (PCA), using the Unscrambler v.9.7 software (CAMO, Norway). Correlation analysis was performed by using Statgraphics Plus v. 5.1 package (Statpoint Technologies, Inc. Warrenton, VA 20186, USA).

3.4 RESULTS AND DISCUSSION

3.4.1 Definition of Shelf Life by Survival Analysis

Generally, survival analysis is a collection of statistical procedures for data analysis for which the outcome variable of interest is the time until an event occurs (Kleinbaum 1996). The problem of analysing time to event data arises in a number of applied fields, such as medicine, biology, public health, epidemiology, engineering, economics, and demography (Klein and Moeschberger 1997). The survival analysis approach was used in the shelf life estimation to process the data coming from the consumer acceptability test; in other words, this is a method that evaluates the time at which an event of interest occurs taking into account the presence of censored data (Hough et al., 2003).

The variable T was assumed as the storage time at which the consumers reject the sample. The rejection function $F(t)$ can be defined as the probability P to reject a product before time t, i.e., $F(t) = P(T)$.

Censoring was defined as follows: at a given storage time t two possible answers could be given by the panelists: (a) the sample was perceived as acceptable, indicating that it would be rejected beyond time t, thus the data is right censored; (b) the sample was perceived as unacceptable, indicating that consumer would start rejecting the product before time t, thus the data is left censored. Since each consumer evaluated only one sample, no interval censoring was present.

Usually, rejection times are not normally distributed; on the contrary their distribution is often right-skewed.

For the data under study, the following standard distributions were compared: smallest extreme value, normal, logistic, Weibull, log-normal, and log-logistic. Details about each of these distributions can be found in the literature (Klein and Moeschberger 1997; Meeker and Escobar 1998). To date, there are no statistical tests to compare the goodness-of fit of different parametric models used for interval-censored data. Therefore, visual assessment of how parametric models adjust to the nonparametric estimation is the common practice in choosing the most adequate model (Hough et al., 2003). An alternative and more objective approach to estimate the best fitting is proposed by Hough (2010). This is an empiric system to define the best equation to fit the experimental data. The loglikelihood test proposed by Meeker and Escobar (1998) allows the goodness of fit comparison of two models, one of which (the null model) is a special case of the other (the alternative model). The test is based on the likelihood ratio, which expresses how many times more likely the data are under one model than the other. Lower this value better will be the fitting of the model on the experimental data. The model that gives the lowest loglikelihood would be the best.

Once the likelihood function is formed for a given model, specialized software can be used to estimate the parameters that maximize the likelihood function for the given experimental data.

As reported in the Table 3.1 the Weibull equation has the lowest value indicating the best fitting among the applied equations. In many cases the distribution that follows the consumer product rejection in studies on food is the Weibull distribution (Calligaris et al. 2007, Araneda et al, 2008; Cadelli and Labuza 2001).

Table 3.1. Value of loglikelihood test

Equation	Value
Lognormal	56.63
Exponential	67.99
Loglogistic	57.72
Weibull	52.68

The Weibull equation (3.1) was reported below, in this equation $F(t)$ was expressed as acceptability function:

$$F(t) = e^{-\left(\frac{t}{\alpha}\right)^\beta} \quad (3.1)$$

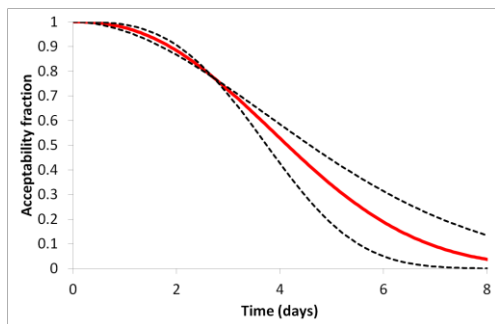
where α is the scale parameter of distribution and β is the shape parameter of the distribution (Gacula and Kubala, 1975).

The Weibull parameters were calculated (Table 3.2; $R^2=0.9998$) and shown in Figure 2.1. By reading the graph in Figure 2.1, taking into consideration a 50% consumer acceptability (vertical axis), the corresponding shelf life can be read on the horizontal axis. Calculated shelf life was estimated with 95% confidence intervals.

Table 3.2. Equation parameters of Weibull distribution with 95% of confidential interval.

	α	β
Average:	4.83	2.37
Lower:	4.21	3.11
Upper	5.55	1.80

Figure 3.1. Interpolation of experimental data using the Weibull equation with confidential interval (95%).



The shelf life assessment at 50 % of acceptance was around day 4.1 ± 0.7 for the sample stored in PET trays. In most studies the sensory shelf life of minimally processed vegetables has been estimated considering the time necessary to reach an arbitrary score of 50% of the scale used to evaluate a certain sensory attribute (Piagentini et al., 2005; Li et al., 2001; Zhou et al., 2004). Comparing this result in terms of shelf life value with assessments presented in literature, this criterion seems strict enough to assure the products' quality at the end of its shelf life.

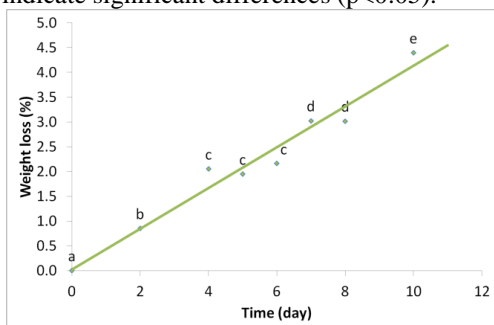
3.4.1.1 Chemical and physical analyses during storage

The physical and chemical analyses were performed to determine the changes in raspberries quality, using the following quality indices: Visual mouldy berries, colour -in terms of Hue angle-, weight loss and firmness of raspberries.

The raspberries during storage lost quickly the weight due to metabolic activity (i.e. respiration and transpiration rates) of fruits (Figure 3.2). However, in this study, weight loss was not over the

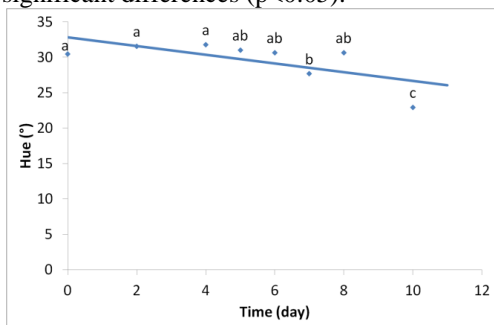
marketability limit reported as 6–8% (Haffner et al., 2002; Nunes, Emond & Brecht, 2003) but the loss can contribute to define a worse appearance to consumer.

Figure 3.2. Weight loss changes of raspberries stored in traditional packaging. Different letters indicate significant differences ($p < 0.05$).



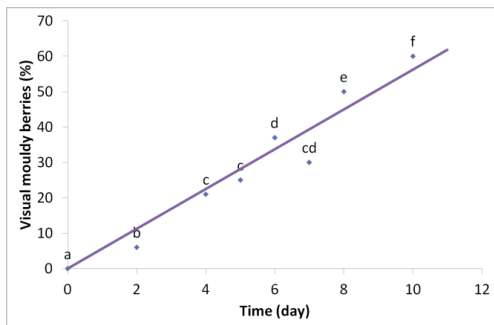
The colour, showed a slightly reduction in terms of Hue angle during storage (Figure 3.3). The colour of the fruits became more red due to the over-ripening and senescence.

Figure 3.3. Hue changes of raspberries stored in traditional packaging. Different letters indicate significant differences ($p < 0.05$).



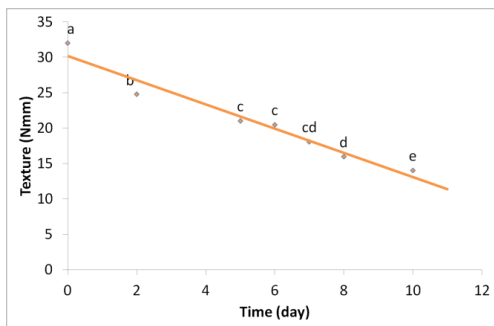
The mould growth on berries during storage is one of the most important limiting factors. In fact as reported in literature the *Botrytis* infection in the small red fruit has the potential to cause up to 50% loss (Hertog et al., 1999; Wszelaki and Mitcham, 2003). This parameter was considered unacceptable when 5% of the berries were visibly affected by mycelium growth (Hertog et al., 1999; Sanz et al., 1999). In this study the limit was reached quickly (around 2 days) and confirms the assumption that this parameter has an important role during storage.

Figure 3.4. Visual mouldy berries of raspberries stored in traditional packaging. Different letters indicate significant differences ($p < 0.05$).



Firmness is a main quality attribute that is critical in determining the acceptability of fruits and vegetables. It is convenient to define quality as the composite of intrinsic characteristics that differentiate units of the commodity - individual pieces of the product - and to think of acceptability as people’s perceptions of and reactions to those characteristics (Abbott and Harker, 2013). In this study the firmness decreases quickly reaching values around 16 Nmm at the end of storage (Figure 3.5).

Figure 3.5. Firmness of raspberries stored in traditional packaging. Different letters indicate significant differences ($p < 0.05$).



3.4.1.2 Correlation between consumer acceptability and quality indices

In order to find the most useful indicator that could be associated to the consumer acceptance, a correlation analysis between the predicted percentage of consumer acceptability (F%) and the chemical and physical indices was carried out (Table 3.3). Soluble solids, acidity, and dry matter were scarcely correlated with the predicted percentage of consumer acceptability ($R < 0.70$). By contrast, the consumer acceptability was strongly correlated with firmness properties suggesting this variable to be potentially available as critical indicator as reported in Table 3.3.

Table 3.3. Correlation coefficients between the percentage of consumer acceptability (F%) and quality parameters of raspberries during storage

Quality indices	R
Firmness (Nmm)	0.999 ^a
Weight loss (%)	0.952
Visual Mouldy berries (%)	0.922
Hue angle (°)	0.913

^ap<0.05.

Firmness is a quality attribute that is critical in determining the acceptability of fruits and vegetables. In the protocol for the determination of the shelf life value, the second step after choosing the critical indicator is the evaluation of the critical limit.

The changes in firmness during storage follow a common degradation as shown in the Figure 3.5. The experimental data were interpolated with a linear regression model identifying the relationship between the critical index and time as a pseudo-zero order kinetic (Oduse and Danny, 2012). The linear regression can be expressed as follows:

$$A_t = k * t + A_0 \tag{3.2}$$

Where: A is Firmness (Nmm), t is storage time (day), k and A₀ are regression parameters as reported -2.03 and 31.95 respectively (0.99 R²).

By substituting in Eq. (3.2) the independent variable t defined in survival shelf life, the following relationship can be obtained (Eq. 3.3):

$$A = k * \alpha * [- \ln(F(t))] / \beta + A_0 \tag{3.3}$$

Where: A is Firmness (Nmm), t is storage time (day), k and A₀ are regression parameters, α is the scale parameter of distribution and β is the shape parameter of the distribution (Hough, 2009).

This equation allows defining the firmness limit related to the end of lifetime of raspberries; taking into consideration the industrial policy, a food company can choose to expose to more or less risk by selecting a certain percentage of consumers rejecting the product.

By using the Eq. 3.3 with the defined parameters (k, α , β , A₀) the estimated firmness value was calculated in correspondence of 50 % of consumer rejection.

The value of the firmness using this equation corresponds to 23.4±1.4 Nmm taking into account a 95% of confidence level.

The last step consists in applying the pseudo-zero kinetics to define the t at which the initial value of firmness reaches its critical limit (Eq. 3.4). In this case this time correspond to the shelf life value (Manzocco and Lagazio, 2009).

$$SL = \frac{A_0 - A}{k} \tag{3.4}$$

Where: A is the acceptability limit of firmness chosen on the basis of 50% of consumer rejection, A₀ is the initial value of the parameter and the k is the rate of firmness decrease.

The shelf life value, taking into consideration the 95% as confidence level resulted in 4.1 ± 0.7 days. This lifetime value is in the same range reported in literature, taking into consideration the same temperature of storage, which was stated between 2-3 days by Hardenburg et al. (1986) and 5 days by Nunes et al. (2003).

3.4.2 Definition of Shelf Life by Multivariate analysis

Multivariate analysis (MVA) is based on the analysis of more than one statistical outcome variable at a time. This technique can be used to assess the shelf life value considering many different parameters and taking into account the effects of all variables on the responses of interest.

One of the most useful techniques based on this approach is the Principal Component Analysis (PCA), which aims at finding a new set of axes in multivariate space that better describe the structure in the data set. These new axes are called Principal Components (PC) and are built by linear combinations of the original variables (Malinowski., 1991; Wold et al., 1987).

Following the steps defined by Pedro and Ferreira (2006) study the multivariate approach was performed.

Firstly a matrix was carried out collecting in the columns the results of physical and sensorial analyses while rows represent the time at which the results were obtained. In this matrix 3 replicates of the raspberries analysis performed along 2 years for different batches of samples were taken into account. This structure of the columns is necessary in order to keep samples spread in a single multivariate space which would reveal time dependence in the PCA; the variables present different scales, and the auto-scale procedure was performed to obtain to obtain the X_a matrix. The columns of X_a have means equal to zero and unit variance (Eq. 3.5):

$$X_a = \frac{X_{n,k} - \bar{X}_k}{S_k} \quad (3.5)$$

where X_k and S_k are, respectively, the mean and the standard deviation of the elements of the k-th column of X and X_a , k and $X_{n,k}$ are typical elements of X_a and X . n is the number of points in time where evaluations were conducted.

Secondly build up shelf-life charts (PC scores vs. time) for the first R PC and identify the scores which are time-related and for each of the time-related PC, identify their reaction order and determine the kinetic parameters using the PC scores as properties.

Thirdly to identify gathering the cut-off criteria for the score time-related PC using the loading values of the PCA should be used the following procedure (Eq. 3.6):

- 1) Place the reference values for each property into the x vector and pre-process it using the parameters determined in equation 3.5 to obtain X_a .
- 2) Use the loadings matrix to calculate the cut-off criteria that is the maximum acceptable scores for each time-related PC:

$$T_{crit} = X_a * L_m \quad (3.6)$$

where X_a is the row vector of reference values and L_m is the loadings matrix of the time-related PC for storage condition (Pedro and Ferreira 2006).

The changes in raspberries quality indices stored in traditional packaging can be described by using few quality indices with respect to all the parameters tested in this study. The analysis of loading of the original matrix established that the loading that have an impact more than 0.3 was collected to use it in the final matrix and to estimate the global quality indices. This selection was carried out to determine the best quality indices that describe the changes in the berries during storage. In fact

only 5 indices were used to evaluate the shelf life of berries using PCA approach respect to all parameters measured in this work as reported in Table 3.4. The selection defined the weight loss, firmness, visual mouldy berries, visual colour evaluation and visual acceptability as the main quality indices to describe the quality decay.

As reported in literature, the berries can loss quickly the weight during storage due to metabolic activity (i.e. respiration and transpiration rates) of fruits. In our study, weight loss was around 3% which is lower than the marketability limit (6–8 %; Haffner et al., 2002; Nunes, Emond & Brecht, 2003). In this study the firmness limit found (23 Nmm) in previous section has been reached and define an important limit to describe the lifetime value. As described above the firmness decrease its value following the pseudo-zero order kinetic.

The mould growth during storage on berries is one of the most important limiting factors. In fact as reported in literature the *Botrytis* infection in the small red fruit with the potential to cause up to 50 % loss (Hertog et al., 1999; Wszelaki and Mitcham, 2003). This parameter was considered unacceptable when 5 % of the berries was visibly affected by mycelium growth (Hertog et al., 1999; Sanz et al., 1999). In this study the limit was reached quickly (around 2 days) and confirms the assumption that this parameter has an important role in the storage. The acceptability limit has been individuated in literature as the best combination between the economic and marketable sustainability of berries and the limit was fixed at 50 % (Labuza et al., 1999). This index can contribute to define an overview assessment of berries quality. The berries reached this value during storage after 4-5 days. The visual colour reached the limit expressed as score 3 in a 1-5 scale (Hertog et al., 1999) after around 6-7 days of storage.

Table 3.4: The matrix of quality indices used in the PCA to identify the best parameters to estimate the shelf life value

SAMPLE	Weight loss (%)	TSS (Brix°)	a*	b*	Hue angle (°)	Croma (C*)	Acidity (g/100g)	Firmness (Nmm)	Visual acceptability (%)	Visual moulded berries (%)	Visual colour evaluation (score)	Dry matter (%)
A0	0.0	10.1	23.7	14.6	31.5	27.8	2.0	25.1	100	0	1.00	13.3
A2	1.6	10.9	24.1	14.8	31.5	28.3	1.7	21.3	85	6	2.00	12.6
A4	2.1	10.0	26.7	16.5	31.8	31.4	1.6	19.4	35	21	2.00	12.1
A7	3.0	9.2	26.6	16.2	31.3	31.2	1.5	18.1	20	30	3.00	11.4
B0	0.0	8.0	27.2	17.7	33.1	32.4	1.5	30.9	100	0	1.00	10.9
B2	0.8	8.7	27.1	16.5	31.4	31.7	1.4	28.3	79	8	2.00	12.4
B6	2.2	8.3	27.0	16.0	30.7	31.4	1.3	20.5	33	37	3.00	11.8
B8	3.0	8.9	27.5	16.3	30.7	32.0	1.3	15.9	6	70	3.00	12.4
C0	0.0	9.2	36.3	18.3	26.8	40.6	1.7	70.4	100	0	1.00	11.4
C5	1.9	10.0	32.3	13.5	22.6	35.0	1.6	34.6	45	5	2.00	11.1
C7	3.0	10.6	30.2	13.5	24.0	33.1	1.4	26.6	20	13	3.00	11.8
C10	4.4	9.9	28.2	11.9	22.9	30.6	1.5	22.4	0	45	4.00	12.0

Limits used for each quality index, taking into consideration only selected parameters, are reported in Table 3.5

Table 3.5. Limit values for different quality parameters to identify the PC critic

Variable	Unit	Limit	References
Visual mouldy berries	%	5	Hertlog et al., 2009
Weight loss	%	6	Robinson et al. 1975
Visual colour evaluation	Score	3	Nunes et al. ,2003
Firmness	Nmm	23	Adobati et al. 2015
Visual acceptability	%	50	Lareo et al., 2009

To identify the general limit to estimate the shelf life value a useful method was used as described in the work of Pedro and Ferreira (2006). The matrix of the data from the 5 selected parameters was analyzed in the principal component analysis.

In PCA two principal components have accounted for 82% of the variation in the original data set, a reasonable amount of information considering the intrinsic variability of the original properties. Figure 2.6 shows the scores chart for the first two PC. Samples are labelled with their respective times (t) and the name of batch in order to visualise the correlation of each PC with time-related degradation. The PC1 is time structured, in fact the samples were distributed along this line in function of the time while the PC2 describes the variation among samples A-B versus C. The batch C is different from the others due to the higher firmness values as described by “texture” in the loading plot (Figure 3.7). At the end of the storage the sample C was characterized by a higher weight loss and higher visual colour evaluation. The samples A and B at the end of the storage were described by a higher mould growth than in sample C.

The loadings chart in Figure 3.7 reveals the key attributes responsible for product degradation. It can be seen that those variables which increase in time have positive PC1 loadings whilst those which decrease present negative values. The Texture and the Visual acceptability have shown negative values defining a decrease trend during storage, while Weight loss, Visual colour score and the Visual mouldy berries have shown positive values defining an increase trend during berry storage.

Figure 3.6. Scores plot of Principal Component

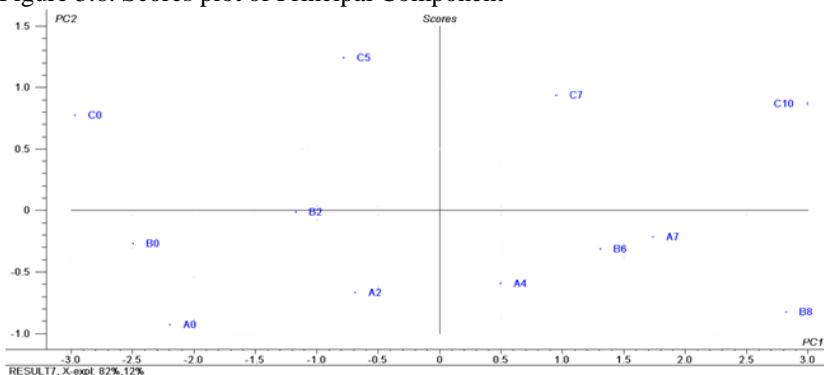
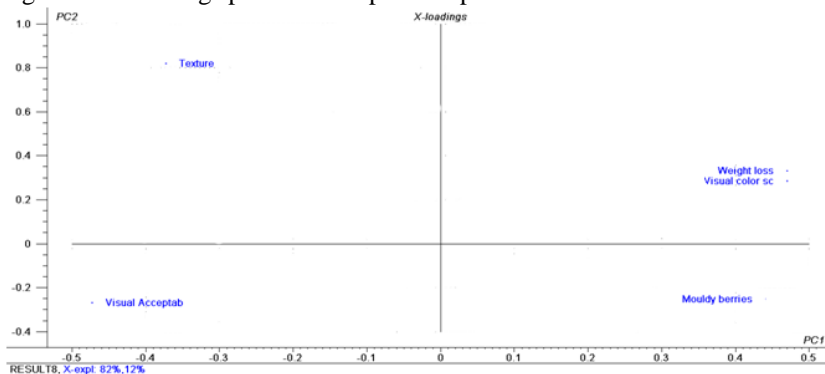


Figure 3.7. Loadings plot of Principal Component

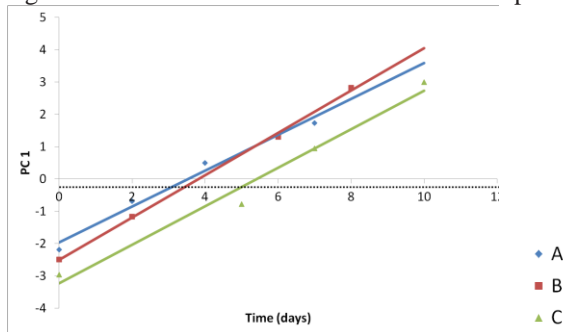


The multivariate approach can use all parameters that have effect on the quality changes in raspberries. Considering the property of time-structure of PC1 it was possible to define an overall degradation reaction with pseudo-zero-order kinetic (Table 3.6) for different batch samples .

Table 3.6. Fitting of linear equation the PC1 data in order to obtain the pseudo-zero order kinetic.

Batch	a	b	R ²
1	0.6551	-2.5047	0.9987
2	0.5558	-1.9697	0.9797
3	0.5964	-3.232	0.9782

Figure 3.8. PC 1 versus time for each batch samples



Applying the reference values reported in Table 5 for each quality attribute and using the equation (3.6), a critical PC1 score value of -0.2 was obtained (refer to Figure 3.8), which corresponds to 4 ± 1 days of storage. Also in this case the shelf life value of raspberries evaluated with multivariate approach is in the range between 3-5 days as defined in the above approach.

3.5 CONCLUSIONS

In this study the two methodologies to assess the shelf life value were applied. The survival analysis approach can predict the shelf life of foods on the basis of the consumer acceptance requirements and the changes of quality indices by an easier, faster and “cheaper”

methodology (no need for expensive instrument). Once the instrumental analyses have been assessed and correlated with proper survival analysis results, further time-costing consumer tests can be skipped and the detection of such indices may be routinely applied to evaluate shelf life in the industry quality control programs (Manzocco and Lagazio 2009). Although in many works (Curia et al., 2005; Calligaris et al., 2007; Giménez et al. 2007) this procedure is useful due to the standardization of food in terms of formulation and process, in this kind of food (small red fruit) the variability among batches could have a negative effect on the estimation of shelf life using this approach. The factors of fruit variability derive from the cultivar but also from environmental condition of picking, growing and post-harvest cooling. Therefore the definition of instrumental limit correlated with the consumer acceptance can have an inaccurate evaluation due to the changing in the critical index that defines the overall quality. It is not sustainable, in terms of time and cost, to repeat with a large numbers of people the test for each cultivar or each environmental condition. There is the need to find a more representative and suitable indicator to estimate the shelf life, taking into consideration the variability of this type of fruit, in terms of the cultivar and the batch.

This problem can be resolved successfully determining the actual shelf-life by using the multivariate technique (Principal Component Analysis). By gathering the kinetics of main parameters into a single variable, it provided a reduction of the number of calculations performed, giving the information on what are the main parameters affecting berries degradation during cold storage. The critical cut off was calculated using more than one reference value and this technique can contribute to increase the reliability of shelf life value calculated. This is extremely advantageous since, in industry, scientists, managers and marketers often struggle to define individual criteria for each measured property.

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4- PASSIVE AND ACTIVE PACKAGING IN MASTER BAG SOLUTIONS TO EXTEND THE SHELF LIFE OF RED RASPBERRIES (*RUBUS IDAEUS L.*)

4.1 INTRODUCTION

Small red fruits and in particular raspberries (*Rubus idaeus L.*) have very short shelf-life due to physiological aspects such as high respiration rate, loss of firmness, mould susceptibility and tissue breaking down. In fact, the most common reasons of customer complaints are the expired, smashed and leaky fruits (Nunes et al., 2009). The high perishable characteristics can contribute to an important food loss and waste along the supply chain up to 75% from field to retailer and also 3-5% during distribution (WRAP, 2011).

The growers and the researchers have been studying different techniques to extend the shelf-life of fruit including the atmosphere modification controlling the oxygen and carbon dioxide levels around the fruits. This technique is useful for the transport and storage of berries (Kader, 1989). The atmosphere modification in packaged fruit can follow two main strategies:

- a) matching the correct film permeability and the fruit respiration rate in terms of oxygen consumption and carbon dioxide production in order to produce a steady condition inside the package (Kader et al. 1989)
- b) establishing the proper conditions extending the shelf life of fruits by means either of packaging modified atmosphere or by using active devices (e.g. carbon dioxide emitters and oxygen scavengers). In the latter case, the devices are inserted into the packaging and they modify actively the atmosphere, more quickly and better than the previous system (Agar et al., 1999; Robinson and Fellman, 1993).

The use of oxygen absorber and carbon dioxide emitters, in combination with a specific film permeability, allow to reach the correct oxygen and carbon dioxide concentrations which are recommended for each type of fruits. In particular, for raspberries, these concentrations are between 5-10 kPa for O₂ (Joles et al. 1994) and 15-20 kPa for CO₂ (Beaudry, 1999). In fact, low levels of oxygen can reduce the respiration and decay (Beaudry, 1999), moreover high concentration of CO₂ can contribute to reduce the fungal growth (Brown, 1992), loss of firmness (Jacxsens et al., 2000; Day, 2001) and, to a lesser extent, the respiration rate. Those gas limits can promote the off-flavour production (e.g. acetaldehydes, ethanol and ethyl acetate) and fruit injury (Pesis, 2005).

In this study, the possibility of extending the shelf life of red raspberries (*Rubus idaeus L.*) using active packaging solutions were investigated, after the definition of the most suitable plastic material to be used as master bag. Critical indicators and cut-off criteria were also investigated and data were used in pointing out the time at which the raspberry lifetime ended in order to evaluate the potential extension of shelf-life due to different packaging solutions. The final aim was to estimate the role of a new packaging technology in reducing environmental impact along the supply chain, whilst taking into account the benefits of the food loss reduction derived from the actual shelf-life extension. Moreover, the study on active packaging compared with traditional and passive atmosphere solutions from a whole perspective could really contribute in improving the active packaging technologies and making the food supply chain more efficient.

4.2 MATERIALS AND METHODS

4.2.1 Fruits

Red raspberries (*Rubus idaeus L.*) cv. Erika, originated from North Italy were provided by a distributor in Milan the day following the harvest and transported to the laboratory where they were immediately stored in a dark cold chamber (5±1°C, 70% RH). At this stage, the fruits were packed in macroperforated PET trays (125 g, dimensions: 14x9x5 cm) closed with PET cover that

represented the commercial unit. In order to simulate the real conditions, fruits were maintained in their original PET trays without sorting.

4.2.2 Packaging solutions and Storage

4.2.2.1 Passive atmosphere packaging solution

Table 4.1 describes the packaging materials used in the experimental plan, including the gas permeability for oxygen transmission rate (O₂TR), carbon dioxide transmission rate (CO₂TR) and water vapour transmission rate (WVTR).

Table 4.1: Characteristics of plastic films used in the master bag form

Code	Material	Thickness (μm)	O ₂ TR ($\text{ccm}^{-2}\text{day}^{-1}$)*	CO ₂ TR ($\text{ccm}^{-2}\text{day}^{-1}$)*	WVTR ($\text{gm}^{-2}\text{day}^{-1}$)**
A	LDPE	500	500	2600	1.1
B	PA/PE** *	150	20	40	3.4
C	LDPE	25	4000	30000	21.7

* 23°C-0 % RH; ** 38°C-90 % RH., *** Polyamide/Polyethylene

4.2.2.2 Active atmosphere packaging solution

For the experimental plan, fruits in their macro-perforated PET trays were packed inside a master bag containing carbon dioxide emitter and oxygen scavenger sachets.

A master bag unit made by LDPE film was used as packaging unit. The characteristics of the bag are the follow: 25 μm thickness, 30x35 cm dimensions, oxygen transmission rate at 23°C and 0%RH equal to 4000 $\text{cc m}^{-2}\text{day}^{-1}$, carbon dioxide transmission rate at 23°C and 0%RH equal to 30000 $\text{cc m}^{-2}\text{day}^{-1}$, water vapour transmission rate at 38°C and 90%RH equal to 21.7 $\text{g m}^{-2}\text{day}^{-1}$.

Inside the master bag unit, two macro-perforated PET trays, containing 125g of berries each one, were inserted and before sealing, a defined volume of compressed dry air (moisturized by using distilled water applied onto paper towels), one oxygen scavenger (FreshPax® CR4, Multisorb Technologies Inc., Buffalo, NY, USA), and a different number of pre-activated carbon dioxide emitters (BioFresh®, Multisorb Technologies Inc., Buffalo, NY, USA; nominal capacity of 500 cm^3) were added to the master bag.

Table 4.2 summarizes the combinations used in this set. The control sample was represented by raspberries stored in the commercial PET tray.

Table 4.2. Solutions tested in the experimental plan

Code	S/UFV* ($\text{cm}^2*\text{cm}^{-3}$)	Number of O ₂ scavengers	Number of CO ₂ emitters
A	1.077	1	2
B	1.077	1	3
C	0.506	1	3
D	0.506	1	4
E	0.506	1	5

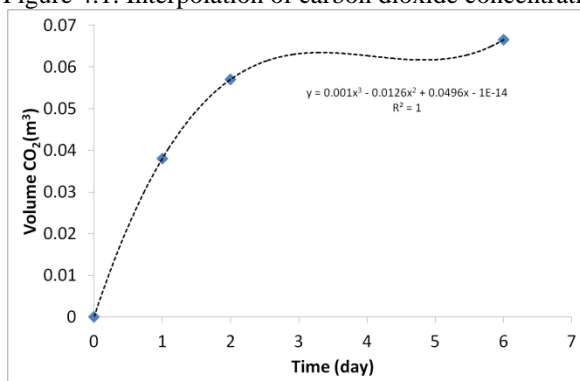
* UFV: unfilled volume inside master bag

4.2.3 Physical and chemical analyses

Head space measurement: Headspace gas composition was periodically sampled by means of a gas-tight syringe, putting an adhesive septum on the film before opening. Oxygen and carbon dioxide were detected and quantified by a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector (TCD) set at 105 °C and a steel column (2 m x 6 mm CTR I Alltech, Milano, Italy). The GC oven was set isothermally at 50 °C.

To better manage the evolution of carbon dioxide inside the master bag in the response surface methodology, the volume of carbon dioxide was used as the response. The volume of carbon dioxide was estimated by determine the area below the best curve that interpolates the experimental points of carbon dioxide concentration in master bag multiplied by the % with the head space volume (0.004m³) as show in Figure 4.1. The results were expressed as m³ of carbon dioxide produced over the storage time.

Figure 4.1. Interpolation of carbon dioxide concentration evolution by best fit simple curve



Respiration rate: Apparent respiration rate (RR) of the raspberries was measured at 5°C using the closed system method. The measurements were carried out in triplicate. Berries and the jars were equilibrated for 1 h at 5°C. Samples (100 g) were then placed in air in 0.5 L glass jars and tightly covered with metal caps equipped with silicone sampling ports. Headspace gas was periodically sampled (20-30 min) by means of a gas-tight syringe. Oxygen and carbon dioxide were detected and quantified by a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector and a steel column (2 m × 6 mm. CTR I Alltech), until the CO₂ level inside the jars reached 5%. Respiration rate was calculated from the linear regression of O₂ and CO₂ concentrations measured during the time of experiment and it was expressed as ml kg⁻¹ h⁻¹.

$$RRO_2 = \frac{\Delta[\%O_2] * V}{(\Delta t * 100 + M)}$$

$$RRCO_2 = \frac{\Delta[\%CO_2] * V}{(\Delta t * 100 + M)}$$

Where:

V= Head space volume, ml

M= Mass of product, kg

t= Time, hours

Percentage of rejected berries: Physically damaged and mouldy berries were visually estimated at each sampling time and the results were expressed as percentage of rejected berries respect to

total berries inside each tray. In particular, raspberry fruits showing surface mycelia development were considered decayed.

Weight loss: The net ($W_{\text{raspberries}}$) and gross (W_0) weights of each package unit (PET tray) before and after the storage in master bag were recorded at day 0 with a technical balance (MP-3000 Chyo Balance corp., Japan) and the gross weight were recorded over the storage time. The weight loss percentage was obtained as follows:

$$\%WLOSS = (W_0 - W_t)W_{\text{raspberries}} * 100$$

Where:

W_0 : weight of raspberries at time 0 (initial), expressed in g

W_t : weight at raspberries at a specific time, expressed in g

Superficial colour of berries: At each sampling time, superficial colour of the berries (L^* , a^* and b^* parameters) was measured on 30 fruits taken from three different packages by an handheld Tristimulus colorimeter (Konica Minolta CR-300, Tokyo, Japan) with a diameter 8 mm, 2° standard observer and a C as illuminant source. Before each measurement, the apparatus was calibrated on the Hunterlab color space system using a white ceramic tile (Minolta calibration plate, $Y = 92.6$, $x = 0.3136$, $y = 0.3196$). Colour was described as Hue angle (H° , expressed as $\arctg(b^*/a^*)$) and Chroma (C, expressed as $(a^{*2} + b^{*2})^{1/2}$) indexes.

Colour by clustering: Many approaches to image segmentation have been proposed over the years (Pal and Pal, 1993; Bhanu and Rarvin, 1987) but clustering is one of the simplest, and has been widely used in segmentation of grey level images (Coleman and Andrews, 1979). The aim of this method is to define the minimization of the sum of squared distances between all points and the cluster centre. k-means algorithm was the method to resolve the clustering procedure of the images. The calculation steps were described by Tou and Gonzalez (1974).

In this study the collected images were processed to transform RGB space into the CIEL*a*b* colour space at the beginning. The data coming from this conversion were analysed by k-means algorithm to define the number of clusters in which split the collected image. In the analysis, the a^* and b^* parameters and 3 clusters were used as clustering factors. One of these clusters represented the background of the image while the others defined the two different colorations of raspberries. The analysis was performed by MATLAB® v. R2012b (MathWorks, Natick, MA, USA).

Texture: Texture of raspberries was determined using a dynamometer (Zwick Roell Instrumental Z010, Zwick GmbH & Co. KG, Ulm, Germany) by a single compression test on single berry (modified method from Sousa et al., 2007). At least 30 berries were evaluated at each time. The berry was positioned under the probe plate (80 mm diameter) and compressed to 60% deformation using a load cell of 10 kg (100 N), at 2 mm/s test speed and with 5 g pre-load. Firmness of samples was evaluated as energy at 60% deformation (Nmm), which corresponds to the labour needed to compress the berries to 60% of initial height.

Volatile compounds: Volatile compounds were assessed using a SPME-GC-MS technique. The raspberries were homogenized with a commercial blender (Sc 300 N Black & Decker Inc. New Britain CT, USA) for 10 seconds (Buttery, 1987) and then frozen at -20°C . Twenty grams of this mixture was thawed overnight at 4°C before analysis. 2-Methyl-1-pentanol (99%, Sigma-Aldrich, St. Louis, MO, USA) was added as internal standard (IS) and stock solution (500 mg/L) was prepared in methanol. Three gram of thawed samples was placed in a 20 ml vial and 1.5 ml of distilled and filtered water containing the IS (0.6 mg/kg) was added.

Extraction was performed as follows: SPME fiber with PDMS-DVB-CAR phases (50/30 μm film thickness); incubation 10min at 40°C; extraction 40 min at 40°C. The analysis of volatile compounds was carried out using Perkin-Elmer Autosystem XL gas chromatograph equipped with a TurboMass selective detector (Perkin-Elmer Inc., Waltham, MA, USA). The analytes were thermally desorbed at 250°C for 10 min in splitless mode (at -0.5min with 0 ml/min of flow and at 2 min opened with 20 ml/min). The Helium column flow was 1ml/min. Separation was achieved using DB-WAXTER column (60m x 0.25mm x 250 μm film thickness, Agilent, Santa Clara CA, USA). The oven temperature was programmed at 45°C for 3 min, then ramped to 70°C at rate 2°C/min, to 230 °C at rate 6°C/min and, held at final temperature for 6 min. Standard EI mode was used at 70eV. The total mass ion chromatography was obtained from 35 to 300amu. System software control and data management/analysis were performed through TurboMass 5.4.2 software (Perkin-Elmer Inc.). Compounds were identified through mass spectra and comparison of their retention index with the pure standards.

Volatile quantification:

External quantification was carried out with the same equipment described previously, using standard solutions of the main off-flavor compounds: ethyl acetate and ethanol, 0,5- 11 $\mu\text{l/kg}$ and 10-2300 $\mu\text{l/kg}$ respectively in methanol (Sigma-Aldrich, St. Louis, MO, USA).

4.2.4 Statistical analysis

Data were statistically evaluated by one-way ANOVA and multiple range test (Tukey method) with Statgraphics Plus v. 5.1 package (Statpoint Technologies, Inc. Warrenton, VA, USA). Significant differences among treatments were determined.

4.2.5 Multivariate approach using Principal Component Analysis

Following the steps defined by Pedro and Ferreira (2006) study the multivariate approach was performed.

Firstly a matrix was carried out collecting in the columns the results of physical and sensorial analyses while rows represent the time at which the results were obtained. In this matrix 3 replicates of the raspberries analysis performed along 2 years for different batches of samples were taken into account. This structure of the columns is necessary in order to keep samples spread in a single multivariate space which would reveal time dependence in the PCA; the variables present different scales, and the auto-scale procedure was performed to obtain to obtain the X_a matrix. The columns of X_a have means equal to zero and unit variance (Eq. 4.1):

$$X_a = \frac{X_{n,k} - \bar{X}_k}{S_k} \quad (4.1)$$

where X_k and S_k are, respectively, the mean and the standard deviation of the elements of the k-th column of X and X_a , k and $X_{n,k}$ are typical elements of X_a and X . n is the number of points in time where evaluations were conducted.

Secondly build up shelf-life charts (PC scores vs. time) for the first R PC and identify the scores which are time-related and for each of the time-related PC, identify their reaction order and determine the kinetic parameters using the PC scores as properties.

Thirdly to identify gathering the cut-off criteria for the score time-related PC using the loading values of the PCA should be used the following procedure (Eq. 4.2):

- 1) Place the reference values for each property into the x vector and pre-process it using the parameters determined in equation 4.1 to obtain X_a .
- 2) Use the loadings matrix to calculate the cut-off criteria that is the maximum acceptable scores for each time-related PC:

$$T_{crit} = Xa * Lm \quad (4.2)$$

where Xa is the row vector of reference values and Lm is the loadings matrix of the time-related PC for storage condition (Pedro and Ferreira 2006).

The changes in raspberries quality indices stored in traditional packaging can be described by using few quality indices with respect to all the parameters tested in this study. The analysis of loading of the original matrix established that the loading that have an impact more than 0.3 was collected to use it in the final matrix and to estimate the global quality indices. This selection was carried out to determine the best quality indices that describe the changes in the berries during storage

In order to analyse the results from a multidimensional point of view, the obtained data were analyzed by Principal Component Analysis (PCA) using the Unscrambler v.9.7 software (CAMO, Norway).

4.2.6 Experimental Plan

In the first part of the work, the influence of different films (A, B and C, described in Table 4.1) on raspberries shelf-life using the passive solution was studied. Master bags containing two PET sale units, heat-sealed without gas flushing were produced using those films. Raspberries were subsequently stored at 5 ± 1 °C (70 %RH) and their quality monitored after storage days 2, 4 and 7.

After the selection of the best master bag material, in the second part of the work the active solution using carbon dioxide emitters and oxygen scavenger into the master bags in comparison with passive solution was investigated. At this step, two trays were inserted inside a master bag, with active devices as explained in Table 4.2 and different ratio between surface of the film and unfilled volume. In addition, in this case, master bags were heat-sealed without gas flushing, subsequently stored at 5 ± 1 °C (70 %RH) with quality monitored after 2, 6, 8 and 13 days of storage.

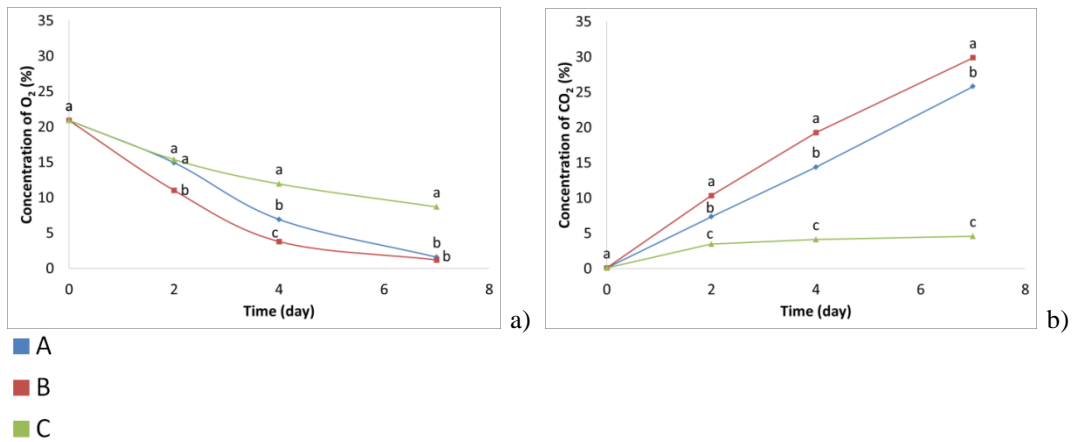
4.3 RESULTS AND DISCUSSION

4.3.1 Passive atmosphere packaging solution

The respiration rate was determined on the fresh fruits at 5°C and values obtained were $RRO_2=20$ ml O_2 $kg^{-1} h^{-1}$ and $RRCO_2=19$ ml CO_2 $kg^{-1} h^{-1}$. In general, high respiration rates are associated with poor quality after harvest: the respiration rate determined on raspberries was in the little high for raspberry (Perkins Veazie and Nonnecke, 1992), with an respiratory quotient $RRCO_2 / RRO_2$ equal to 0.97. This value can be associated with an aerobically respiration due to the consumption of carbohydrates (Saltveit et al., 2014).

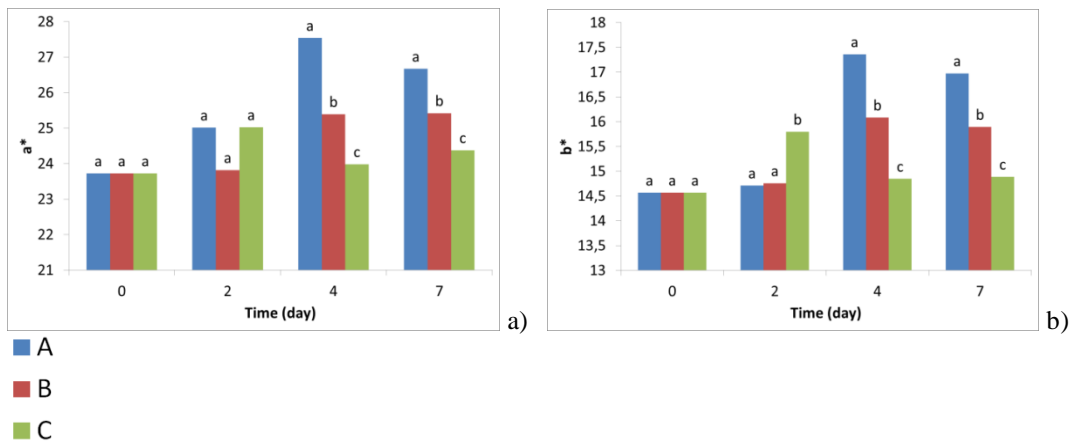
During storage in master bag, using different packaging materials, the decrease in O_2 and the increase in CO_2 were registered for all the samples (Figure 4.2). Master bags A and B (medium and high oxygen and carbon dioxide barrier, respectively) indicated a faster accumulation of carbon dioxide and a rapid decrease in oxygen reaching values under 5 % during storage time. These conditions could induce the cells to switch from aerobic to anaerobic metabolism (Joles et al., 1994). Differently, sample C (the lowest gas barrier film) presented slower oxygen decrease reaching an optimal concentration around 10 % after 4 days in master bag, while CO_2 reached a roughly 5 % concentration maintained until storage day 7.

Figure 4.2. Headspace gases evolution in master bags made with materials A, B and C (a: oxygen; b: carbon dioxide). Different letters for the same time indicate significant differences ($p < 0.05$).



The senescence process of raspberries is well known to cause colour changes, especially on the red (positive values of a^*) and blue (negative values of b^*) coordinates of the CIELab colour space (Haffner et al., 2002). The colour trends of samples stored in master bag A (medium gas barrier master bag) were very similar, whereas those stored in the lowest gas barrier master bag (C) maintained the initial colour until day 7, probably due to the respiration processes deceleration and the avoidance of anaerobic conditions (Figure 4.3). An intermediate behaviour between samples stored in film A and C was found for samples stored in film B (high gas barrier).

Figure 4.3. Colour changes in terms of a^* and b^* of raspberries stored in master bag A, B, and C. Different letters for the same time indicate significant differences ($p < 0.05$).



Raspberries packed with medium and high barrier films (A-B) did not present any mould development since the level of carbon dioxide was above the mould toxicity limit (Joles et al.; 1994); however, they were affected by the softening and breakage of drupes (fruits) reaching

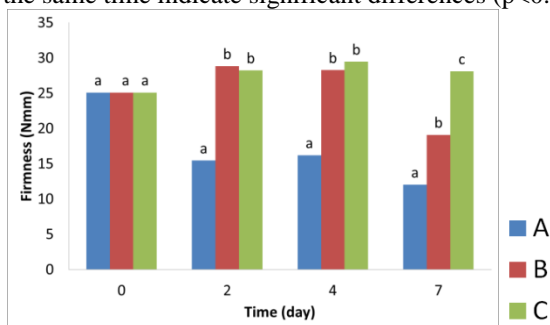
percentage of rejection higher than 15% after 7 days of storage. In raspberries storage the most satisfactory behaviour was found by using the lowest barrier master bags (C) with maximum rejection of 10 % at the final stage of the test.

Fruit firmness is defined as the ability of fruit to maintain integrity, shape and avoid the release of juices. Consumers are able to assess fruit texture through a simple visual evaluation before purchasing; if the product does not meet their requests in term of firmness and colour consumers reject it.

The use of a master bag had a positive impact on raspberries firmness (Figure 4.4), especially when the film with the greatest gas permeable feature was used (C); this is probably due to a lower respiration rate influenced by the gas evolution (Aday et al. 2011). In fact, the latter solution was able to maintain the initial quality of berries in terms of firmness until the end of the test (day 7), whereas the control samples were strongly degraded after only 2 days of storage.

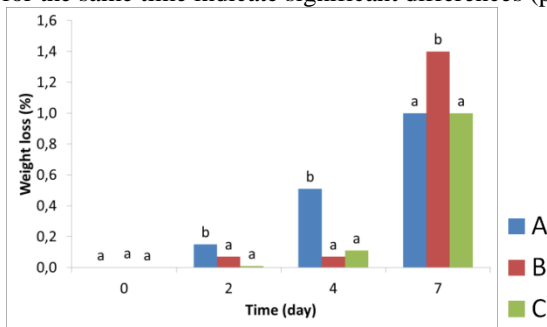
In addition, the films with higher WVTR seemed to affect the fruits softening process: as shown in Figure 2, a quickly change occurred in samples stored in film A presenting the lowest WVTR ($1.1 \text{ g m}^{-2} \text{ day}^{-1}$). The presented quality indices showed that the use of master bags film with low barrier to oxygen, carbon dioxide and water vapour provided a passive atmosphere modification able to maintain the quality of raspberries longer than the traditional packaging in air, up to at least 7 days storage.

Figure 4.4. Firmness changes of raspberries stored in master bag A, B, and C. Different letters for the same time indicate significant differences ($p < 0.05$).



The master bag, can protect the raspberries against the dehydration of fruit due to the transpiration. The low water vapour permeability of films allowed to maintain a high level of relative humidity in the head space, thus this system (passive packaging) generating a weight loss closed to the 1,5 % at the end of the storage (Figure 4.5). This value was lower than the one showed in the traditional solution (3%).

Figure 4.5. Weight loss changes of raspberries stored in master bag A, B, and C. Different letters for the same time indicate significant differences ($p < 0.05$).



4.3.1.1 Determination of shelf life by Multivariate approach

All the data obtained from the experimental trials were analysed by means of Principal Component Analysis. Five attributes were considered at each time of analysis for each packaging solution (A-B-C), respect to all parameters measured in this work as reported in Table 4.3.

The selection defined the weight loss, firmness, visual mouldy berries, and concentration of oxygen and carbon dioxide were the main quality indices to describe the quality decay.

The first of two principal components explain the 73% of variability. A separation of the samples according to the storage conditions is shown on the scores plot (Figure 4.6), where the number beside each point represents the storage time in days. In particular, samples were distributed along PC1 according to the storage time. The advantage of using the loadings plot is that it visually presents the correlations between variables. For instance, the number of mouldy berries, the weight loss and the carbon dioxide concentration are strictly correlated. Loadings revealed also the weight of attributes responsible for product degradation (Figure 4.7).

Fruits stored in plastic film with high or medium OTR were characterized by an increase of carbon dioxide as showed in the Figure 4.2. This condition generated high percentage of rejected berries due to the damages caused by the toxic effect of carbon dioxide on the berries surface and on the metabolism of fruit.

The samples C and B were characterized by higher firmness than the sample A. This parameter seems to split the samples in two groups following the PC2. This evaluation seemed correlated with the lowest water vapour permeability of the film where the sample A was stored. When in pack RH is very high (>95%) the proliferation and spread of microorganisms can occur during the storage (Rahman et al., 2007).

Figure 4.6. Scores plot of the first two Principal Components

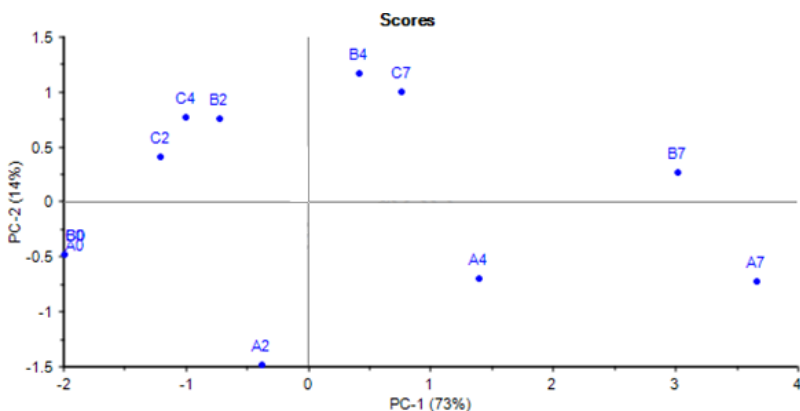
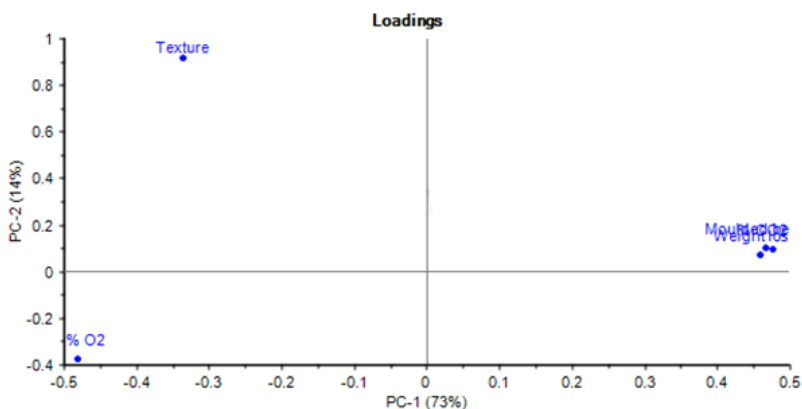


Figure 4.7. Loadings plot of the first two Principal Components



The multivariate approach uses all parameters that have effect on the quality changes in raspberries. PC1 was time structured and therefore this is the most suitable PC for estimating shelf-life parameters, as it can be seen in Figure 4.6. In other words, the overall degradation reaction followed a pseudo-zero-order kinetic, defining a linear equation for all the packaging solutions with different parameters as reported in Table 4.4.

Table 4.4. Pseudo-zero-order equation parameters.

Solution	a	b	R ²
A	0.8135	-1.9679	0.9989
B	0.7109	-2.1298	0.9897
C	0.3783	-2.0863	0.9396

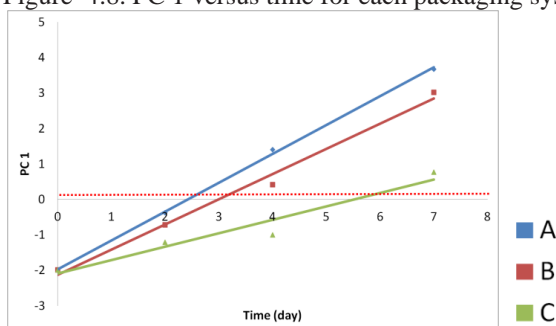
The limit used for estimating the critical PC for passive packaging solutions was reported in Table 4.5. With the multivariate approach, using five quality indices simultaneously, the efficacy of the critical indicator determination can be improved respect to the classical protocol that identifies only one limit for each parameter. When the quality attributes of foods like small red fruits are quickly

altered for the effect of several degradative processes, the multivariate approach based on a single cut-off criterion could be a useful tool to estimate the shelf life value.

Table 4.5. Limit values for different quality parameters to identify the PC critic for passive packaging solution

Indices	Unit	Limit	References
Visual Rejected Berries	%	10	Sanz et al., 1999
Weight loss	%	6	Robinson et al., 1975
Texture	N*mm	23	Adobati et al.,2015
Concentration of O ₂	%	5	Joles et al., 1994
Concentration of CO ₂	%	25	Watkins, 2000

Figure 4.8. PC 1 versus time for each packaging system



Applying the reference values for each quality attribute expressed above and using the Eq. 4.2, a critical PC1 score value of 0.12 was obtained (see Figure 4.8), which corresponds to berries stored in solution A at 2.6 days, in solution B at 3.2 days and in solution C at 6 days. The last value suggests that the berries packaged in LDPE 25 μm with high OTR ($4000 \text{ cc m}^{-2}\text{day}^{-1}$) had an higher shelf-life compared to fruits stored in “traditional” solution (4 days) as defined in the first chapter and reported in literature (Giuggioli et al. 2015).

The multivariate approach defined successfully the shelf-life of raspberries stored in a passive packaging system. The material selected in this part of the study (material C) was used in the experiment described in the next paragraphs and related to the active packaging solution.

Table 4.3: The matrix of quality indices used in the PCA to identify the best parameters to estimate the shelf life value

Samples	Visual Moulded berries (%)	Weight loss (%)	Texture (Nmm)	Concentration of O₂ (%)	Concentration of CO₂	TSS (Brix°)	l*	a*	b*	Hue (°)	Chrome (C*)	Acidity (%)
A0	0,00	0,00	25,08	20,90	0,03	10,09	30,25	23,72	14,565	31,23	27,88	2,01
B0	0,00	0,00	25,08	20,90	0,03	10,09	30,25	23,72	14,565	31,23	27,88	2,01
C0	0,00	0,00	25,08	20,90	0,03	10,09	30,25	23,72	14,565	31,23	27,88	2,01
A2	3,00	0,15	15,43	14,93	7,34	9,87	29,73	25,01	14,71	30,36	29,03	1,92
B2	4,00	0,07	28,81	11,02	10,33	10,03	29,22	23,82	14,76	31,45	28,05	1,98
C2	6,00	0,01	28,22	15,37	3,48	10,17	28,76	25,02	15,80	32,03	29,62	1,7
A4	12,00	0,51	16,19	6,91	14,38	9,99	30,40	27,54	17,36	31,92	32,61	1,80
B4	7,00	0,07	28,25	3,79	19,27	10,3	29,55	25,39	16,08	32,25	30,07	1,93
C4	5,00	0,11	29,44	11,93	4,12	10,2	28,19	23,98	14,85	31,56	28,23	1,65
A7	22,00	1,00	12,02	1,58	25,79	10,13	31,87	26,67	16,98	32,31	31,64	1,67
B7	10,00	1,40	19,08	1,19	29,87	10,43	28,20	25,42	15,89	31,90	30,00	1,78
C7	14,00	1,00	28,09	8,68	4,57	10,03	28,94	24,38	14,89	31,18	28,59	1,57

4.3.2 Active atmosphere packaging solution

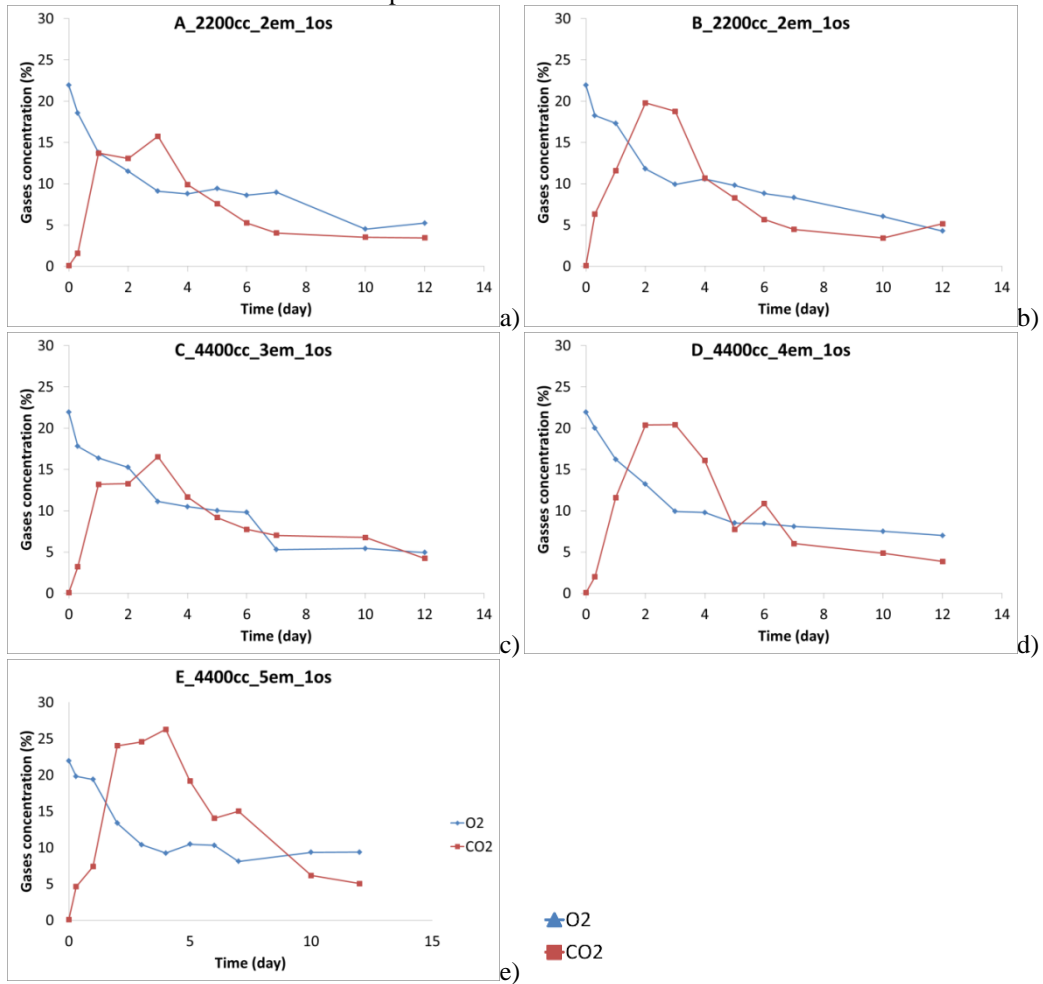
The respiration rate was determined on the fresh fruits at 5°C and the obtained values were $RRO_2=13 \text{ ml O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ and $RRCO_2=18 \text{ ml CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$. In general, high respiration rates are associated with poor quality after harvest: the respiration rate determined on raspberries was lightly high (Perkins Veazie and Nonnecke, 1992), with a quite aerobically respiratory quotient ($RRCO_2 / RRO_2=1.36$) but with a metabolism that prefers the organic acid to produce energy (Saltveit et al., 2014).

The gas evolution inside the master bags is shown in Figure 4.9a-d for oxygen and carbon dioxide, respectively. For both the gases, the curve's shape is quite different with respect to that obtained in open air: in that case, a steady oxygen concentration of O_2 and CO_2 is achieved when fruit O_2 uptake and CO_2 production rates are equal to the rates of O_2 and CO_2 flux through the film (Joles et al., 1994).

During storage in master bag, using different combinations between the number of carbon dioxide emitters and the ratio between film surface and unfilled volume, different changes in the headspace and also in the quality changes were obtained.

In the Figure 4.9 the combinations B, D and E highlighted a faster accumulation of carbon dioxide, in some cases higher than 20%. These conditions can induce the cells to switch from aerobic to anaerobic metabolism, generating damaged and off-flavour (Joles et al., 1994). Differently, sample stored in the combinations A and C presented a lower carbon dioxide value in the first part of the storage, reaching the optimal concentration (10-15%; Joles et al., 1994) to reduce the mould growth without side-effect of the fruit quality. In these two cases the fast and short period of carbon dioxide exposure (from 1 to 4 days) have contributed to reduce the fruit metabolism and the spoilage.

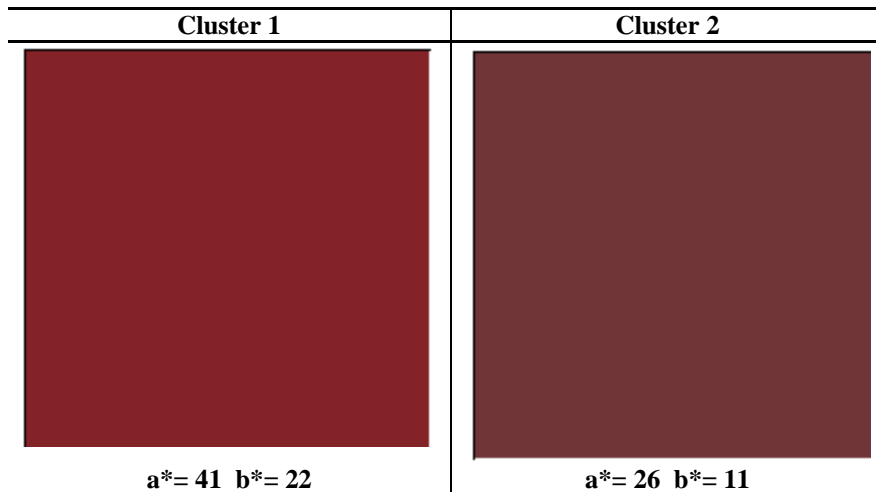
Figure 4.9 Gases concentrations evolution of CO₂ and O₂ inside the master bag for different number of active devices and head space volume



The senescence process of raspberries is well known to cause colour changes, especially on the red (positive values of a*) and blue (negative values of b*) coordinates of the CIELab colour space (Haffner et al., 2002). Using the clusterization technique it was possible to define the change from the red colour associate to an appreciate colour (ripe fruit) to purple indicating an over-ripe/senescent product.

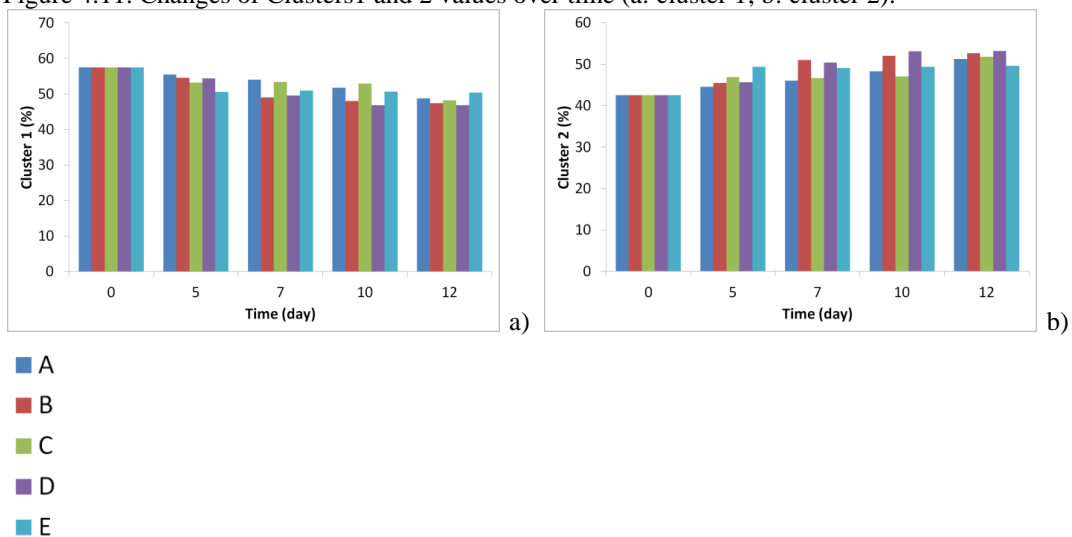
The two cluster was defined by the a and b colour value as represented in the Figure 4.10.

Figure 4.10. Graphical representation of the clusters derived from the imagine clusterization and the respective coordinate a* and b*.



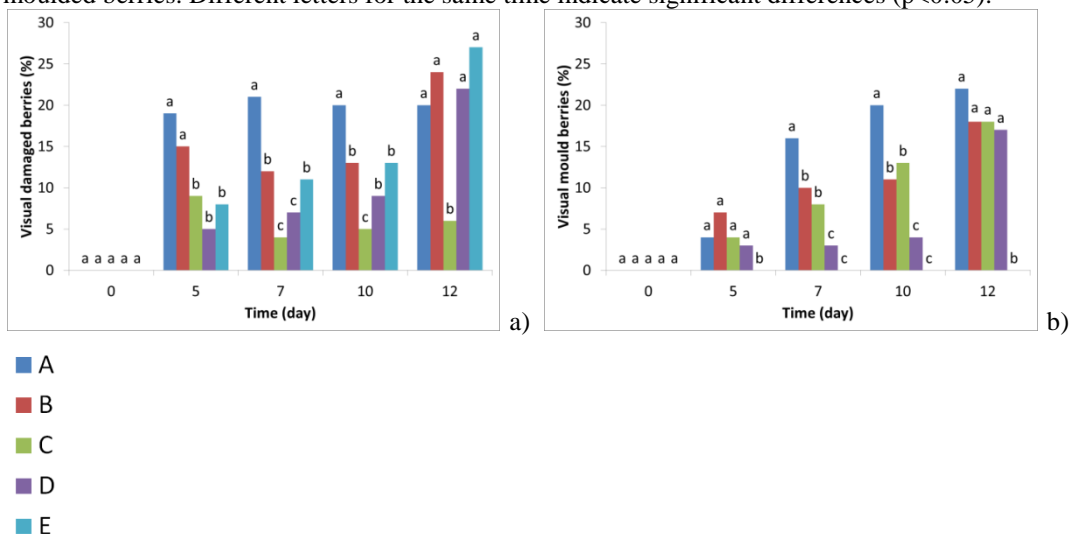
As expected, all samples showed a changes of the colour linked to the ripe fruit (cluster 1) in function of an increase of colour correlated with the over-ripening (cluster 2). In fact the area on the berries surface occupied by the appreciate colour (cluster 1) showed a changes in the colour developing unacceptable value. As reported by Sanz et al., (1999) if the surface raspberry shows more than 1/3 in damaged, defined as over-ripe colour, the consumer rejects the product. For all samples the surface represented by ripe colour decreased during storage from about 60% up to 45%, on the contrary the surface that showed the unacceptable colour increase from 40% to 55% (Figure 4.11).

Figure 4.11. Changes of Clusters1 and 2 values over time (a: cluster 1; b: cluster 2).



High percentages of visual rejected berries characterized sample A due to the incorrect evolution of gases inside masterbag, low concentration of carbon dioxide and too low oxygen concentration (< 5% Joles et al. 1994; Figure 4.12 a, b). Sample with high level of carbon dioxide (B,D,E) showed a high level of damaged berries and also mould growth except in sample E. In this case the highest value of CO₂ (>25%) at 5 days of storage leads to inhibit the mould growth. As define by Haffner et al., (2003) the moulds don't growth above 20 % of carbon dioxide.

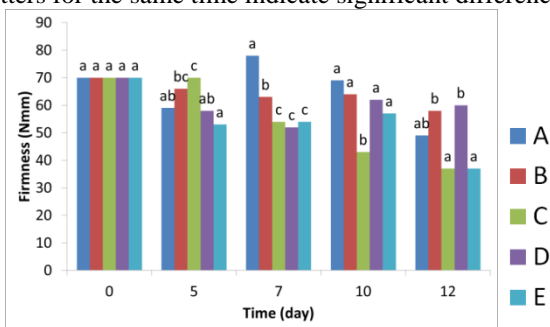
Figure 4.12. Visual rejection of berries for each packaging solution – a) damaged berries; b) moulded berries. Different letters for the same time indicate significant differences (p<0.05).



Fruit firmness is defined as the ability of fruit to maintain integrity, shape and avoid the release of juices. Consumers are able to assess fruit texture through a simple visual evaluation whilst purchasing; if the product does not meet their requirement in term of firmness and colour consumers reject it.

The changes in the texture of different packaging solution showed a decrease of firmness in the samples C and E, in the first case due to the normal senescence of fruits and in the second case due to the toxic effect of carbon dioxide of products (Figure 4.13). Whereas the sample B and D maintained the firmness level probable due to the effect of high level, but not toxic, of carbon dioxide. Regarding other fruits like strawberries, the short exposure of carbon dioxide can maintain the firmness or in some case increase it (Larsen and Watkins, 1995).

Figure 4.13. Firmness changes of raspberries stored in master bag A, B, C, D and E. Different letters for the same time indicate significant differences ($p < 0.05$).

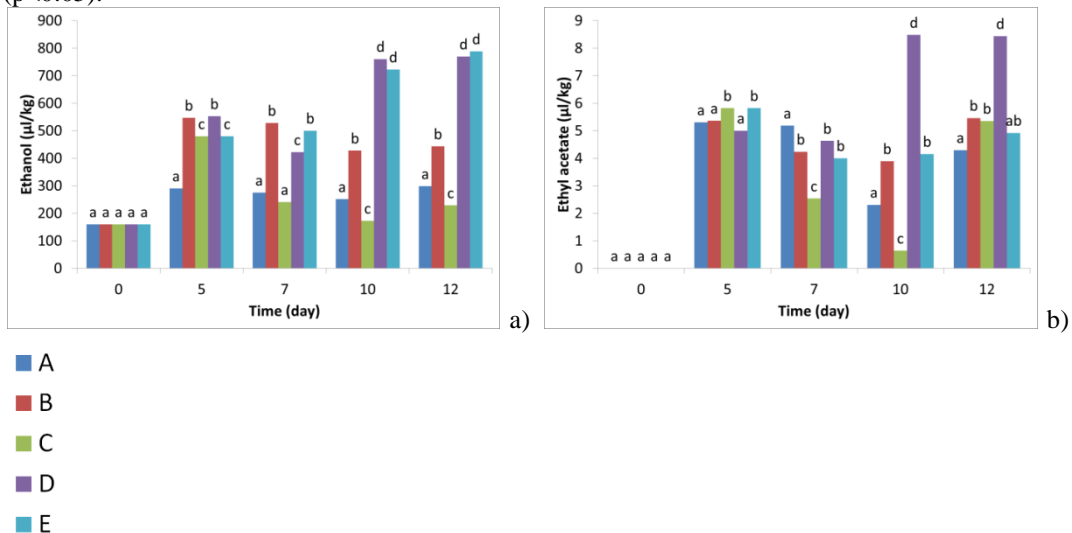


The off-flavour compounds are good indicators to evaluate the changing in the metabolism, especially the ethyl-acetate compound is the most important compound correlated to the consumer perception of off-flavour in the strawberries (Larsen et al., 1999).

It is well known that the appreciation of flavour by the consumer depends on many compounds but some compounds define mostly the unappreciated flavour.

The high concentration of carbon dioxide (Figure 4.9 b,c,d,) inside the master bag in B, D, E samples determined an increase in the ethanol and ethyl-acetate production during storage (Figure 4.14). In master bag C the fruit showed an increase in off-flavour at 5 days of storage due to the changing in the atmosphere respect to the room atmosphere (21% O₂ and 0.03 % CO₂). In accordance with the gas evolution inside the master bag (Figure 4.9), after this short period (5 days) the fruit returns to the aerobic metabolism until 10 days; finally the senescence of fruit and the consumption of oxygen lead to an increase of ethyl acetate that defines the anaerobic condition of fruit (Larsen and Watkins, 1995). The samples stored in master bag with 2 carbon dioxide emitters showed a similar behaviour with the sample C in terms of ethanol production but reaching higher value of ethyl-acetate compound.

Figure 4.14. Volatile compounds (Ethanol (a) and Ethyl acetate (b)) changes of raspberries stored in master bag A, B, C, D and E. Different letters for the same time indicate significant differences ($p < 0.05$).



The master bag, as described in passive packaging solution, can protect the raspberries against the transpiration thanks to the low water vapour permeability; the loss of soluble metabolites can decrease the consumption of sugar and acid to produce energy. The master bag contributes to reduce the weight loss reaching close to the 1 % but extremely lower than the marketable limit set to 6% (Figure not shown)

4.3.2.1 Definition of the shelf life by the Multivariate approach

To define the shelf life value, also for active packaging solution the multivariate approach was used as described from Pedro and Ferreira (2006). This technique can gather the limit of many quality indices to define the time at which the samples don't satisfied the quality requirement.

In the preliminary section, all the collected parameters (Visual damaged and moulded berries, Weight loss, firmness, Cluster 1 and 2, Off-flavour compounds, Oxygen concentration and quantity of CO_2 ; Table 4.6) were used for the berries stored with master bag. Using the loading value from PCA matrix, the selection was performed to identify the best indicators to describe the quality changes in the raspberries and to increase the explained mostly the PC variation.

A total of 10 PCs were retained from the PCA applied to the ratings of the 6 attributes used for quality attributes of raspberries.

The first of two principal components explain the 80% of variability. A separation of the samples according to the storage conditions is shown on the scores plot (Figure 4.15), where the number beside each point represents the storage time in days. In particular, samples were distributed along PC1 according to the storage time. Loadings revealed the weight of attributes responsible for product degradation (Figure 4.16). The fruits stored with E and D are characterized by fermentative off-flavour and high value of carbon dioxide during storage while the samples A, C and B show an increase in damaged and mould during storage.

Figure 4.15. Scores plot of the two Principal Components

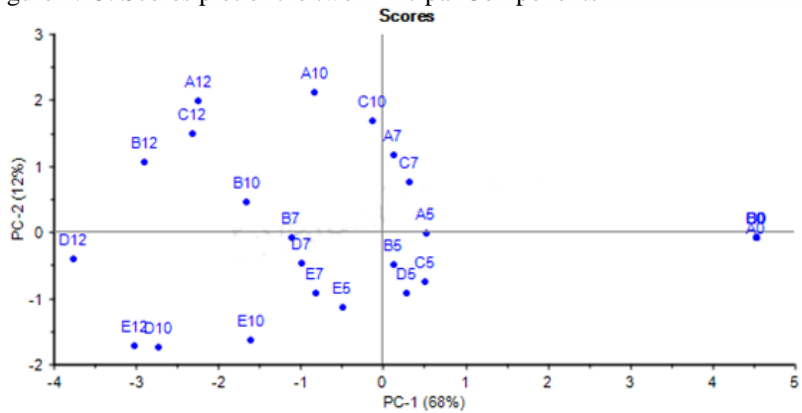


Figure 4.16. Loadings plot of the two Principal Components

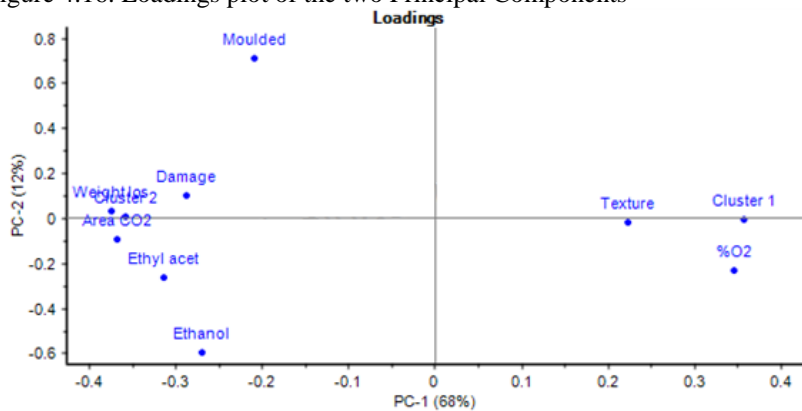


Table 4.6. The matrix of quality indices used in the PCA to identify the best parameters to estimate the shelf life value

Sample	Visual damage berries (%)	Visual moulded berries (%)	Weight loss %	Work (Nmm)	Color Cluster 1 (%)	Color Cluster 2 (%)	Ethanol ($\mu\text{g}/\text{kg}$)	Ethyl acetate ($\mu\text{g}/\text{kg}$)	O ₂ (%)	CO ₂ (%)	Volume of CO ₂ (m ³ *day)
A0	0	0	0	69,95	57	43	160	0	22	0	0.00
A5	19	4	0,66	58,74	55	45	210	5	9	8	0.22
A7	21	16	0,66	77,77	53	45	274	5	9	4	0.27
A10	20	20	0,94	68,91	52	48	251	2	5	4	0.32
A12	20	22	0,96	48,75	49	51	298	4	5	3	0.35
B0	0	0	0	69,95	57	43	160	0	22	0	0.00
B5	15	7	0,63	65,54	55	45	547	5	10	8	0.27
B7	12	10	0,76	62,95	49	51	528	4	8	4	0.32
B10	13	11	0,98	63,56	42	52	427	4	6	3	0.37
B12	24	18	1,01	58,06	47	53	443	5	4	5	0.40
C0	0	0	0	69,95	57	43	160	0	22	0	0.00
C5	9	4	0,46	69,86	53	47	480	6	10	9	0.24
C7	4	8	0,75	53,67	53	47	241	3	5	7	0.30
C10	5	13	0,86	43,21	53	47	173	1	5	7	0.38
C12	6	18	1,06	36,93	48	52	229	5	5	4	0.43

Sample	Visual damage berries (%)	Visual moulded berries (%)	Weight loss %	Work (Nmm)	Color Cluster 1 (%)	Color Cluster 2 (%)	Ethanol (µg/kg)	Ethyl acetate (µg/kg)	O ₂ (%)	CO ₂ (%)	Volume of CO ₂ (m ³ *day)
D0	0	0	0	69,95	57	43	160	0	22	0	0.00
D5	5	3	0,64	58,14	54	46	552	5	9	8	0.29
D7	7	3	0,81	52,00	50	50	422	5	8	6	0.36
D10	9	4	1,08	61,85	47	53	760	8	8	5	0.43
D12	22	17	1,15	59,73	47	53	769	8	7	4	0.46
E0	0	0	0	69,95	57	43	160	0	22	0	0.00
E5	0	0	0,46	52,81	51	44	480	6	10	19	0.32
E7	11	0	0,80	54,34	51	49	500	4	8	15	0.41
E10	13	0	1,09	57,13	51	49	722	4	9	6	0.51
E12	30	0	1,26	37,19	50	50	788	5	9	5	0.56

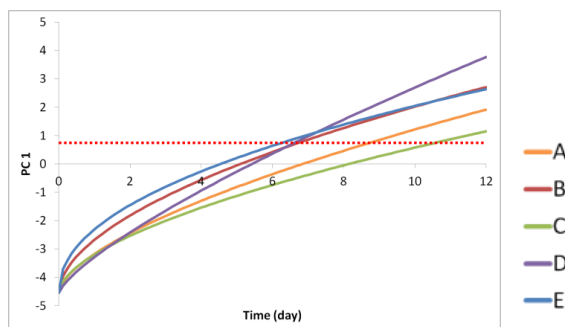
The multivariate approach can be able to use all parameters that have effect on the quality changes in raspberries. Using the property of time-structure of PC1 in function of time, the Figure 4.15 was defined. The overall degradation reaction followed power-law ($y=a+bx^c$) for samples and define the kinetics of degradation for different samples (Table 4.7).

Table 4.7. Fitting of linear equation the PC1 data in order to obtain the power-law model

	a	b	c
A	-4.52	1.34	0.63
B	-4.54	1.86	0.55
C	-4.52	1.32	0.59
D	-4.54	1.25	0.76
E	-4.53	2.22	0.47

The power law is able to explain the degradation of some food components in relation with an isothermal condition (Peleg et al., 2002 and Kong et al., 2007). These different changes in the fruit quality parameters could be explained observing the interaction between the food and the packaging system in terms of the gases permeability and the oxygen and carbon dioxide scavenging and emitting respectively by the active devices inserted into the master bag before bag-sealing. The changes in the raspberries' quality parameters collected in the PC1 are the result of the fast changing of gases composition in the bag headspace and the metabolism of berries, affecting the different changes in the senescence changes.

Figure 4.17. PC 1 versus time for each batch samples.



Applying the reference values for each quality attribute expressed below in the Table 4.7 and using the equation (2), a critical PC1 score value of 0.75 was obtained (refer to Figure 4.17), which corresponds to different shelf-life value for each packaging solution. As expected the solution B, D and E reach a lower value of shelf life, in particular 7.3, 7.0 e 6.9 respectively. The samples A and C reach the best extension reaching 9.3 and 11.3 days of shelf life, respectively.

Table 4.7. Limit values for different quality parameters to identify the PC critic for active packaging solution

Indices	Unit	Limit	References
Visual Damaged Berries	%	10	Sanz et al., 1999
Visual Moulded Berries	%	5	Hertog et al., 1999
Weight loss	%	6	Robinson et al., 1975
Firmness	N*mm	23	Adobati et al., 2015
Colour Cluster 1	%	67	Sanz et al., 1999
Colour Cluster 2	%	33	Sanz et al., 1999
Ethanol	µl/kg	414	Larsen and Watkins, 1995
Ethyl acetate	µl /kg	467	Larsen and Watkins, 1995
O₂ Concentration	%	5	Joles et al., 1994
Volume of CO₂	m ³ *day	100	Almenar et al., 2008

The multivariate approach has successfully defined the shelf life of raspberries stored in traditional packaging. By gathering the kinetics of main parameters into a single variable, it provided a reduction of the number of calculations performed, giving the information on what are the main parameters affecting berries degradation during cold storage. The critical cut off was calculated using more than one reference value and this technique can contribute to increase the reliability of calculated shelf life value.

4.4 CONCLUSION

The aim of this work was to determine the shelf-life of red raspberries and find new packaging solution to extend their shelf-life. The evaluation of the quality indicator using the multivariate approach allowed to define with an high level of reliability the value of shelf-life for berries stored in different packaging solution.

The changes of berries in different environmental conditions don't permit to use the same indicator or the same critical limit for the indicator. Every time the study have been adjusted to evaluate the new packaging conditions and the new changes in the fruit quality, in other words finding the correct approach for each packaging solution.

At the end of the study it can be defined, objectively, the role of packaging system to extend the shelf-life of raspberries. This "new" packaging solution have used the active device to reach quickly the optimal conditions, in terms of oxygen and carbon dioxide, and it has been optimized taking into consideration the link with the fruit metabolism and the gas permeability.

The active packaging solution can allow to store the raspberries at least until 11 days almost three times more than the "traditional" packaging solution that reach a shelf-life value of 4 days. The Passive packaging solution can allow to store the berries until 6 days, thus extending the shelf-life. Finally, the new packaging solution can extend the shelf life and probable reduce the environmental impact of food chain thanks to the reduction of food loss due to the preserved quality of raspberries longer than the "traditional" packaging solution.

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5- COMPARATIVE LIFE CYCLE ASSESSMENT OF RASPBERRIES STORED IN DIFFERENT PACKAGING SOLUTIONS

5.1 INTRODUCTION

Small red fruits and in particular raspberries have very short storage life due to physiological aspects. The production of raspberries (*Rubus idaeus* L.), in Italy consists in 1500 tons per year (FAO, 2012) and ranks 16th in Europe, after the main East European states (Russian Federation, Poland, Serbia). The market, in this sector, has been increasing due to the production differentiation, profitability and sustainability of cultivation in terms of economy and environment preservation (Agronotizie 2008). The high perishable characteristics such as high respiration rate, loss of firmness, mould susceptibility, and breaking down tissues define the most common reasons of customers' complaints (Nunes et al., 2009). These characteristics can contribute to important food loss and waste along the supply chain up to 75% until its arrival at the retailer (Martin et al. 2010) and in particular WRAP study (2011) estimated the loss and waste in different steps of the supply chain in the UK. The loss of these berries during harvest was from 2-20% depending on different factors such as harvest methodology and weather conditions, 2-3 % during the packaging step and 2-3 % at retailer. In addition about 2-5 % can be wasted at storage level due to the inefficiency of cold chain or logistic management.

The growers and the researchers have been studying different techniques to extend the shelf life of fruit including the atmosphere modification to control the oxygen and carbon dioxide levels around the fruits. These techniques are useful to transport and store berry fruits (Kader,1989). The atmosphere modification in packaged fruit can follow two principal strategies: by matching the correct film permeability and the fruit respiration rate of oxygen and carbon dioxide of packaged fruit it is possible to produce a steady-state condition of oxygen and carbon dioxide (Kader et al. 1989) and establish the correct conditions to extend the shelf life of fruits. In the second case the devices (e.g. carbon dioxide emitters and oxygen scavengers) are inserted in the packaging to modify the atmosphere more quickly and better than the previous system (Agar et al., 1990; Robbins and Fellman, 1993).

The use of oxygen absorbers and carbon dioxide emitters, in combination with a correct film permeability, can reach the correct concentration of oxygen and carbon dioxide which are recommended between 5-10 kPa for O₂ (Joles et al. 1994) and 15-20 kPa for CO₂ (Beaudry, 1999). The lower levels of oxygen can reduce the respiration and decay (Beaudry, 1999), moreover the high concentration of CO₂ can contribute to reduce fungal growth (Brown, 1992), loss of firmness (Jacxsens et al., 2000; Day, 2001) and, in lower proportion, reduce the respiration rate.

Using this system it is possible to extend the shelf life of berries define a possible environmental impact reduction. As defined in different works (Williams et al. 2012, Christiansen 2014, Bowling 2013, Fao, 2011) the packaging can contribute to reduce the food loss and the environmental load of the whole system. To measure this possible reduction associated to shelf life extension the Life Cycle Assessment (LCA) methodology was applied to different packaging solutions.

5.2 MATERIALS AND METHODS

The raspberry fruits are cultivated in Europe especially in the East due to the climatic aspect and traditions in those area. In Italy their production is located, in particular, in the Alpine Valley and has grown in recent years. The raspberry production considered in this work takes place in Trentino region (Northern Italy). In this area the pedoclimatic conditions support the small red fruits production (Agnolin 2007).

5.2.1 Goal and scope of application

The goal of this study was to evaluate the environmental impact of production and distribution of fresh raspberry fruits packaged with different methods: “Traditional” sale unit (Base scenario, A), and Passive Atmosphere Modification solution in master bag (B scenario) and Active Atmosphere Modification solution in master bag with devices (C scenario) as two alternative scenarios. The characteristics of packaging solutions are the following:

A) Lidded macro-perforated PET trays containing 125 g of berries, stored in air and considered as “traditional” packaging. This solution has 4 days as shelf life (Chapter 3).

B) Two lidded macro-perforated PET trays containing 125 g of berries inserted into master bags (34cm*25.5cm) made of plastic materials Low Density Polyethylene (LDPE; oxygen transmission rate at 23 °C and 0 %RH equal to 4000 cc m⁻² day⁻¹, carbon dioxide transmission rate at 23 °C and 0 %RH equal to 30000 cc m⁻² day⁻¹, water vapor transmission rate at 38 °C and 90 %RH equal to 21.7 g m⁻² day⁻¹). This solution was referred to as a passive modified packaging solution and has 6 days as shelf life (Chapter 4)

C) Two macro-perforated PET trays (each containing 125 g of berries) inserted into a master bag unit (30cm*35cm) made of LDPE (as described above). Before sealing, one oxygen scavenger (FreshPax® CR4, Multisorb Technologies Inc., Buffalo, NY, USA), and three pre-activated carbon dioxide emitters (BioFresh®, Multisorb Technologies Inc., Buffalo, NY, USA; nominal capacity of 500 cm³) were added to the master bag. All the samples were stored in a cold chamber (5±1 °C; 70±5 %RH). This solution has 11 days as shelf life (Chapter 5).

To compare and evaluate the environmental load of the packaging system, taking into account the shelf life of berries the LCA methodology was applied. Life Cycle Assessment (LCA) is a standardized methodology used for estimating environmental burdens associated with life cycle of products or processes (ISO 14040, 2006). This methodology is considered to be effective for evaluating environmental performance in the agro-food and beverage sector (Roy et al. 2009).

In general in the LCA study in agro-sector (Nalley et al. 2011; Gan et al. 2011; Gonzalez-Garcia et al., 2012) the functional unit (FU) was defined as mass (kg) but in this case, the unit was expressed as a day of shelf life. This choice allows referring the environmental impact to one day of shelf life considering 250 g of raspberries as market quantity. For raspberries production and for packaging environmental impact evaluation the FU was considered as 250 g of product, identifying two sale units.

This work has been carried out from a “cradle to grave” view. The LCA model was carried out by including two subsystems: Crop production (SS1) and post-harvest management of raspberries (SS2).

SS 1 involves the crop cultivation: the system boundary was set from the grower (that also provides for the packaging of fruits in PET trays) to the distribution center, taking into account all of the processes required for cultivation and transport to central distribution. Concerning the crop production all data were referred to the hypothetical 1-hectare plot and a 10 t of berries production in an annual production season during full plant growth, this yield was agreed for literature (Girgenti et al. 2013).

SS 2 involves the post-harvest management of fruits: the system boundary was set from gate of center distribution to consumer home. The phase of consumption is not included in the system, nor the transportation from the supermarket to the consumers' home. However, the disposal of the packaging material is taken into account as municipal management.

5.2.2 LCA inventory

For SS 1 the data were collected from a farm that produces only small fruits and in particular raspberries (70 % of entire production).

The hypothetical hectare of orchard was set using 0.5 m distance from one plant to another in a row and 2.2 m distance between two rows and about 7300 raspberry plants have been implanted in the orchard. The data were collected through questionnaires submitted to technical workers in the farm. The crop production was divided into three steps: Field operations, Fertilizing and Crop protection. The information from workers in the farm was collected and applied to the 1 PET trays of raspberries.

5.2.2.1 Field operations: The operations required for the production were calculated taking into account the surface required to produce 125g of berries (one tray of product), the information is summarized in the Table 5.1.

Table 5.1. Principal field operations concerned in raspberries production analysis

Field operation	Quantity	Unit
Mowing, by rotary mower	0.088	m ²
Tillage, harrowing, by rotary harrow	0.051	m ²
Tillage, ploughing	0.051	m ²
Application of plant protection product, by field sprayer	0.051	m ²
Mulching	0.051	m ²
Irrigation	0.015	m ³
Solid manure loading and spreading, by hydraulic loader and spreader	13.890	g

To reduce the growth of weed a mulching film made by polypropylene was used and the quantity referred to 125 g of raspberries was equal to 292g.

For the fruit production fertilization and plant protection, treatments were applied. The base fertilization was applied as manure in order to improve the organic compounds in the soil, the quantity added every year was estimated in 1 ton per hectare. For the mineral fertilization, ammonium sulphate was applied as nitrogen compound (600 kg per hectare); single superphosphate was applied as phosphate compound (200 kg per hectare) and potassium sulphate was applied as potassium compound (300 kg per hectare; Table 5.2). Concerning the fertilization, the information about nutrient (nitrogen) removal from fruits was taken from De Gennaro work (2012). Emissions due to the fertilizer application were also included in the inventory. Nitrogen emissions (nitrate, ammonia and nitrous oxide) were modeled following the IPCC Guidelines (2006); while phosphate emissions were calculated in accordance with Smil (2000) losses of P equal to 1% of the total applied phosphorus.

Pesticide derived emissions were estimated according to the approach expressed in Ecoinvent 3.0. Using this assumption, the fraction of active substances entering into the soil is assumed to be 100% of the total mass applied quantity (Nemecek and Schnetzer; 2011). The background data for the production of raspberries, the fertilizers, pesticides and field operations were obtained from the Ecoinvent 3.0 database.

Table 5.2. Principal emissions from fertilizers and pesticides in raspberries production

Operation	Compound	Quantity	Unit
Plant protection treatments	Pesticide, unspecified	0.0375	g
	Pyrethroid	0.0028	g
	Potassium bicarbonate	0.0694	g
Base fertilization	Manure	13.89	g
Mineral fertilization	Ammonium Sulphate	8.33	g
	Single Superphosphate	2.78	g
	Potassium Sulphate	4.17	g
Output	Compound	Quantity	Unit
Emission to air	Dinitrogen monoxide	0.02	g
	Ammonia	0.17	g
Emission to water	Nitrate	0.5	g
	Phosphate	0.01	g
Emission to soil	Potassium bicarbonate	0.0694	g
	Copper	0.0208	g
	Lambda-Cyhalothrin	0.0028	g
	Abamectin	0.0187	g

5.2.2.2 Transport

Concerning the transportation of trays, the distance from packaging producer industry (located in Emilia Romagna region) to the producer was 220 km. It was assumed that all the transportations involved a full load trucks. Master bag -film low density polyethylene (LDPE) 25 µm- was transported between local company and the central of distribution (60 km).

For the transport of fruit in the trays from grower to distributional center located in Milan, commercial truck (32 t) was considered (Euro 3). The distance was calculated by Web software (Google Maps, Google inc., Parkway Mountain View, CA) and it was about 240 km. It is assumed that all the transportations involved a full load trucks.

The second SubSystem (SS2) involves the post-harvest management of fruits. The fruits were stored in a cold chamber to maintain the quality of products. In a distributional center the raspberries were delivered to retailer as function of their requests while in the alternative scenarios, in distributional center, the fruits were packed in master bags before storage.

5.2.2.3 Refrigeration

The second SubSystem (SS2) involved the post-harvest management of fruits. The fruits were stored in a cold chamber to maintain their quality for longer times. In a distributional center the strawberries were delivered to retailer as function of their requests, while, in the alternative

scenarios, the fruits could be packed in master bags before storage to extend their shelf life at distributional center.

The products were refrigerated from field temperature, estimated, in the spring-summer condition at 25°C to reach the storage temperature closed 4°C by cooler. In literature the optimal temperature to store the strawberries is -0.5 - 0°C (Cantwell, 2002) but in traditional chamber the fruits were refrigerated from 2 to 4°C (Nunes et al. 2009).

A computational approach was performed to estimate the electrical energy required for cooling the raspberries and to maintain the temperature during the storage (Bonauguri and Miari, 1988). The calculation took into account different heat sources presented in the cooler system: the air inside the refrigerated chamber and the air exchange due to door opening during the fruits movement, the heat coming from walls, ceiling and floor, (considering 25 °C as external temperature) and from fruit metabolism (0.08 W/kg; Sharma et al., 2013), the energy utilized by lights and other devices used inside the cooler. The calculation was determined 4.63 Wh per kg of product.

5.2.2.4 Packaging components

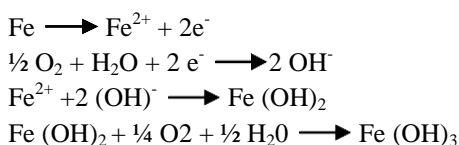
5.2.2.4.1 Active devices

Oxygen scavenger

To estimate the environmental burden of active devices it was used the common formulation found in literature, because the recipe is covered by industrial secret. The oxygen scavengers are self-activate devices and their functionality is performed through the oxidation reaction presented in Equation 5.1 (Schroeder et al. 2001). Usually, the well-known coformulants were the silica gel and sodium chloride (Brody et al., 1995).

The high density polyethylene (HDPE) is the film where these compounds are contained (0.49g).

The iron powder to scavenge 400 cc of O₂ (maximum scavenging for this device, CR4 as reported in M&M) was 1.32 g and 0.648 g of pure water.

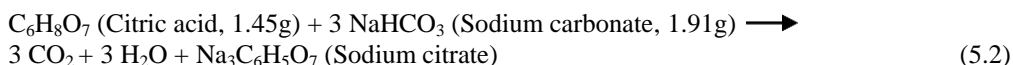


For the silica gel, the absorption of water was estimated at 15 % (w/w) that correspond at 50 % of their maximum absorption value equal to 30 % (Afonso & Silveira, 2005). To absorb the 0.648g of pure water (used in the reaction 1) 4.3416g of silica gel were required.

As well known in the oxygen scavenger the chlorine ion was required as chemical catalyzer of the oxidation reaction: the sodium chloride added was 1.6 g.

Carbon dioxide emitter

For a Carbon Dioxide Emitter, the reaction presented Equation 5.2 was used to determine the weight of each ingredient.



5.2.2.4.2 Tray

The tray was made by polyethilentereftalate (PET) and in the inventory analysis 13g of the raw materials (made in Europe) were considered for its production and the thermoforming energy requirement was defined as 1.12 Wh for each tray.

5.2.2.4.3 Master bag film

The film was made by low density polyethylene and in the inventory 7.72g of film were inserted as quantity, with an efficiency of production from raw material (pellet) to film up to 97.6%. The extrusion energy requirement was defined as 2 kWh/kg of product.

5.2.2.5 End-life

In the end-life step only the packaging disposal was considered. The Lombardy was considered a region where the packaging waste was collected.

Plastic collection

As Grosso et al. (2012) described, the plastic collection is made in two different ways: kerbside collection in the 33% of collection cases while in the remaining cases (67%) using waste containers on road.

In kerbside collection, the management of characteristics is explained in Table 5.3.

Table 5.3. The management kerbside collection

Transporter	Van < 3.5 t	Lorry 16-32 t
Percentage of used in collection	59.4	40.6
Distance	48.8	48.8

Plastic recycle

Before recycling, the plastics have to be selected to remove the undesirable items and unrecyclable plastics. This phase requires per 1 ton of plastic about 26.6 kWh of electricity and 84 MJ of diesel (Grosso et al., 2012). The efficiency of plastic selection system was assumed as 100% due to the high purity of plastic material.

For PET tray, a 95% of recycling was assumed as efficiency of systems and the remaining 5% was collected in the municipal waste. The whole impact generated for PET production was considered as an avoided impact for the system (Levi et al. 2011).

For LDPE bag, a 95% of recycling was assumed as efficiency of systems and the remaining 5% was collected in the municipal waste. In this case, the study described by Rigamonti and Grosso (2009) was used to model the energy and material necessary for recycling the LDPE (Table 5.4).

Table 5.4. Consumption of energy and raw materials used in recycle process of LPDE.

Input for 1 ton of LDPE	Quantity
Electricity for recycle	381 kWh
Electricity for produce the rod	200 kWh
Natural gas	650 MJ
Water	1.78 m ³

5.2.3 Impact assessment

The software SimaPro® 8.0.1 (PRé Consultants bv. Netherlands) was used for the computation of the inventories data. Among the steps defined within the LCA, only the classification and

characterization stages were undertaken (ISO, 14040, 2006). According to other studies concerning the agricultural-packaging systems (Levi et al., 2011 and Perego et al., 2014) ReCiPe Midpoint (H) V1.08 - Europe Recipe H was used and the following categories were selected to evaluate the environmental load of raspberries supply chain: Climate change, Ozone depletion, Terrestrial acidification, Freshwater eutrophication, Marine eutrophication, Human toxicity and Fossil depletion (Table 5.5).

Table 5.5. Impact categories considered in the analysis according to the ReCiPe Midpoint (H) V1.08 - Europe Recipe (Hierarchy) method.

Impact category	Unit
Climate change	kg CO ₂ eq
Ozone depletion	kg CFC-11 eq
Terrestrial acidification	kg SO ₂ eq
Freshwater eutrophication	kg P eq
Marine eutrophication	kg N eq
Human toxicity	kg 1,4-DB eq
Fossil depletion	kg oil eq

5.3 RESULTS AND DISCUSSION

5.3.1 Production

Many authors agree that the food production step is the main factor in the environment load along the supply chain (Peano et al. 2015; Girgenti et al. 2014; and Seppala et al. 2009, Roy et al. 2009). Figure 4.1 reports the results concerning the environmental load of the raspberries production. In the figure, the impacts of 250 g of raspberries (2 trays) can be shared in different phases. The main step that produces a burden is the use of fertilizers due to the run off of the nutrient compounds from soil to water (Muñoz et al. 2010) emission in air (N₂O and NH₃).

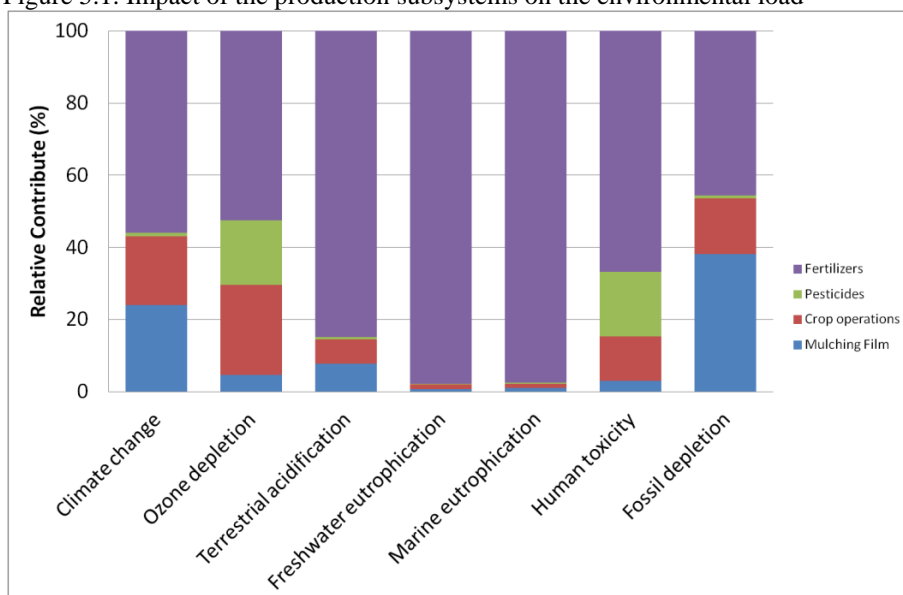
The emissions associated with fertilizer application had a significant impact over all the impact categories and, in particular, in the eutrophication (97%) and in climate change (56%). The film used as mulch had a high effect on the fossil depletion (38%) in relation to the production of the petroleum used to produce the polyethylene.

Table 5.6 was reported the values of the environmental load for different impact categories associated to different steps of the raspberries production. As mentioned above, the fertilizer application had the most important effect on environmental load and this LCI (Life Cycle Inventory) analysis can contribute to improve the knowledge in order to develop a better production system reducing, where it is possible, the loss of nutrient, for example using fertirrigation or more natural fertilizers. Another step to make it possible to improve the crop production is the changing in mulching film material for example using bio-based material as suggested by Girgenti et al. (2014).

Table 5.6. Environmental results for the Raspberry production

Impact category	Unit	Mulching Film	Field operations	Plant protection	Fertilizer
Climate change	kg CO ₂ eq	2.94*10 ⁻²	2.31*10 ⁻²	1.23*10 ⁻³	6.84*10 ⁻²
Ozone depletion	kg CFC-11eq	3.81*10 ⁻¹⁰	2.09*10 ⁻⁹	1.49*10 ⁻⁹	4.38*10 ⁻⁹
Terrestrial acidification	kg SO ₂ eq	1.15*10 ⁻⁴	1.02*10 ⁻⁴	9.63*10 ⁻⁶	1.26*10 ⁻³
Freshwater eutrophication	kg P eq	3.00*10 ⁻⁶	5.21*10 ⁻⁶	6.25*10 ⁻⁷	3.93*10 ⁻⁴
Marine eutrophication	kg N eq	3.16*10 ⁻⁶	4.33*10 ⁻⁶	1.06*10 ⁻⁶	3.28*10 ⁻⁴
Human toxicity	kg 1,4-DB eq	3.90*10 ⁻⁴	1.61*10 ⁻³	2.36*10 ⁻³	8.75*10 ⁻³
Fossil depletion	kg oil eq	1.82*10 ⁻²	7.32*10 ⁻³	3.95*10 ⁻⁴	2.18*10 ⁻²

Figure 5.1. Impact of the production subsystems on the environmental load



The total amount of carbon dioxide emitted for the production of raspberries was agreed with life cycle assessment of the production in Spain of similar fruit such as strawberries (0.35 kg CO₂ eq/kg Williams et al., 2008) and the production developed in Northern Italy (0.053 kg CO₂ eq/125g of berries; Girgenti et al., 2013).

5.3.2 Packaging

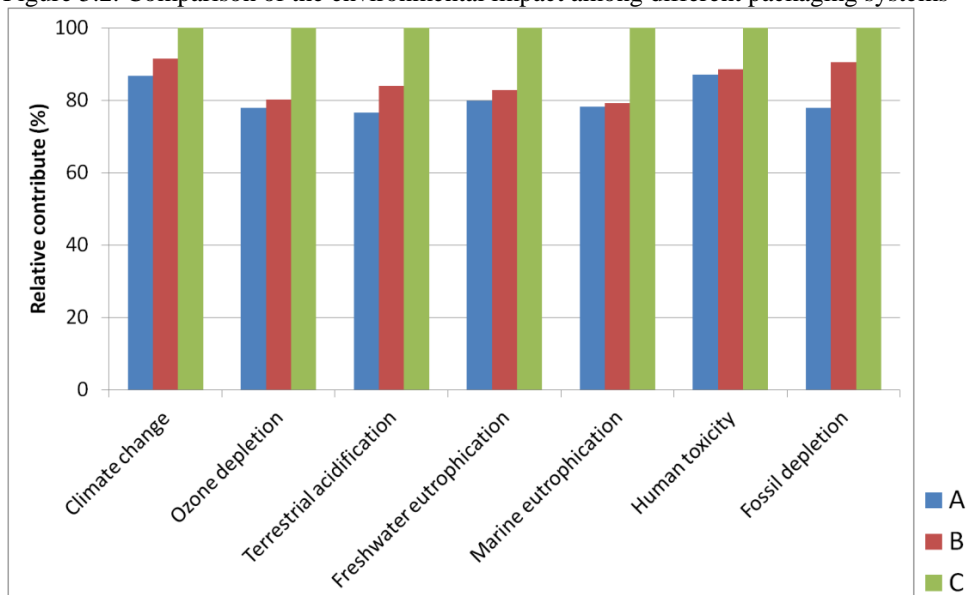
As expected, the analysis of packaging systems demonstrates the increasing of the environmental burden due to the material used and the active compound utilized in the systems called C (Table 5.7).

The absolute amounts of the impacts categories showed similar values but considering the relative impact value (Figure 5.2) the small difference has generated a big share, in particular in ozone depletion, terrestrial acidification, marine and freshwater eutrophication (Figure 5.2). In the results, the contribution of the disposal phase of the packaging showed a big effect due to the collection and recycle process. These results were much higher than Girgenti et al. work reported (2013) on “traditional” tray. This difference was attributed to the different packaging materials considered, in fact in this study the tray was made by PET that had more environmental impact than PE material. Moreover, in the Girgenti et al. (2013) study, the system of disposal was not defined, for this reason the effect of disposal has not contributed as in this study on environmental load.

Table 5.7. Environmental results for different packaging solutions

Impact category	Unit	Pack A	Pack B	Pack C
Climate change	kg CO2 eq	$2,17 \cdot 10^{-1}$	$2,29 \cdot 10^{-1}$	$2,50 \cdot 10^{-1}$
Ozone depletion	kg CFC-11 eq	$5,22 \cdot 10^{-9}$	$5,38 \cdot 10^{-9}$	$6,70 \cdot 10^{-9}$
Terrestrial acidification	kg SO2 eq	$4,92 \cdot 10^{-4}$	$5,39 \cdot 10^{-4}$	$6,42 \cdot 10^{-4}$
Freshwater eutrophication	kg P eq	$3,30 \cdot 10^{-5}$	$3,43 \cdot 10^{-5}$	$4,14 \cdot 10^{-5}$
Marine eutrophication	kg N eq	$1,12 \cdot 10^{-4}$	$1,13 \cdot 10^{-4}$	$1,43 \cdot 10^{-4}$
Fossil depletion	kg oil eq	$4,64 \cdot 10^{-2}$	$5,38 \cdot 10^{-2}$	$5,95 \cdot 10^{-2}$

Figure 5.2. Comparison of the environmental impact among different packaging systems



5.3.3 LCA Comparison among different packaging solutions taking into account the shelf life value of the raspberries packaging solution

The choice of using one day of shelf life as the functional unit means that the impacts were shared along the lifetime. In other words, in this way, it is possible to define a “daily” impact for packaging solutions.

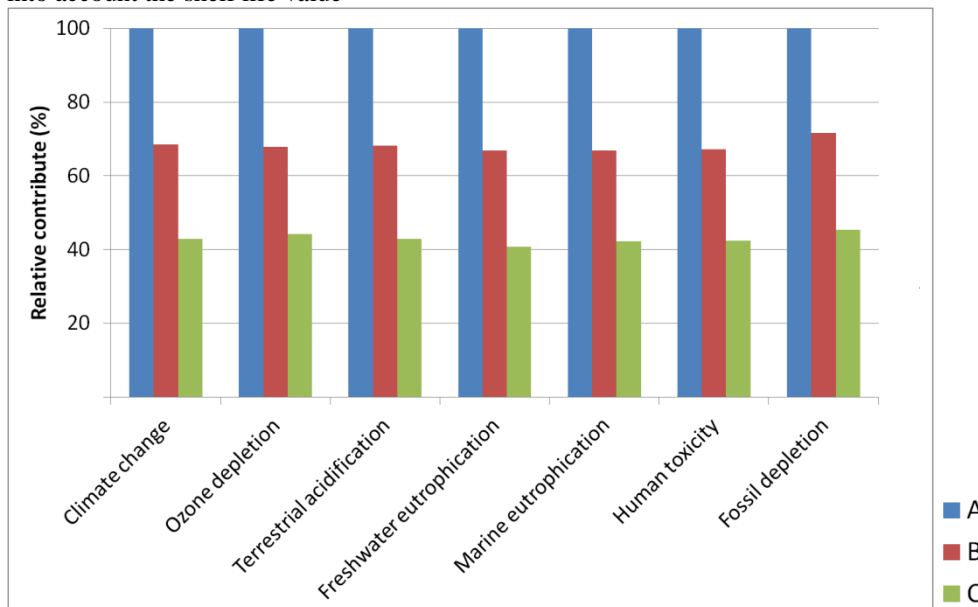
For each impact categories, the differences between base scenarios and alternative scenarios were evaluated. The packaging solution called A has only 4 days of shelf life value and this condition determines the highest daily impact among the packaging solutions studied. Whereas the packaging solutions B and C determine a significative reduction in terms of environmental load up to 55% and 70%, respectively (Figure 5.3). As assumed by different authors (Willliams et al. 2011, Roy et al. 2009, FAO, 2011) a correct packaging can contribute to reduce the overall impact of the system linked also to the reduction of food waste (Almenar et al, 2010). The small differences among packaging solutions for each impact categories were ascribable to the relative impact of the active compound or the master bag film used in the estimation of the environmental impact. These demonstrate that the components of the passive and active atmosphere define a relative high effect on the environmental load of the packaging solution, as showed in Table 5.8.

Table 5.8. Percentage of environmental load referred to the highest value of impact of different packaging solutions

Impact category	Unit	A	B	C
Climate change	kg CO ₂ eq	1.23*10 ⁻¹	8.45*10 ⁻²	5.68*10 ⁻²
Ozone depletion	kg CFC-11 eq	4.30*10 ⁻⁹	2.92*10 ⁻⁹	2.38*10 ⁻⁹
Terrestrial acidification	kg SO ₂ eq	5.52*10 ⁻⁴	3.77*10 ⁻⁴	2.63*10 ⁻⁴
Freshwater eutrophication	kg P eq	1.12*10 ⁻⁴	7.49*10 ⁻⁵	4.70*10 ⁻⁵
Marine eutrophication	kg N eq	1.38*10 ⁻⁴	9.24*10 ⁻⁵	6.00*10 ⁻⁵
Human toxicity	kg 1,4-DB eq	6.69*10 ⁻³	4.50*10 ⁻³	3.44*10 ⁻³
Fossil depletion	kg Oil eq	2.64*10 ⁻²	1.89*10 ⁻²	1.28*10 ⁻²

The differences in the packaging among three scenarios was minimized due to the biggest impact of food production on the entire impact. This means that the packaging could be improved to reduce the environmental load. In fact, in same case, an increase in the environmental load of packaging is needed to reduce the entire impact (Willliams et al. 2011).

Figure 5.3. Comparison of the environmental impact among different packaging solutions taking into account the shelf life value



5.3.4 Tentatively definition of the potential role of the packaging system in reducing the environmental load of food loss

As declared in the FAO work (Gustavsson et al. 2011), one-third of the total amount of food that we produce becomes waste or gets lost during the supply chain. This issue has not only a social and economic component but also an impact on the environmental system, in fact food and drinks production, together with their distribution, represents 20-30% of the total consumption environmental load in the EU (Tukker and Jansen, 2006). Several studies have stressed the importance of increasing the knowledge about the amount of food losses (Carlsson-Kanyama et al., 2002; Davis and Sonesson, 2008) due to the high variation in the literature studies and the lack of identification of the reason why the food waste and loss arise.

Some authors have also highlighted the weight of packaging in the environmental load demonstrating that the environmental impact of packaging is usually relatively small compared to the entire product-package system. The contribution of the whole food chain to the greenhouse gases production, from agriculture to food processing, is predominant. The food production chain and the waste management of packaging are usually 5% of the total environmental loads and in many cases the environmental impact of packaging is as low as 2%, thus having a small effect on the environmental load compared to the food production (Silvenius et al. 2011). The quality attributes appreciated by consumer are often such as to prevent food losses e.g. prevention of leakages, open-dating, protection, declaration of contents, hygiene, instructions, easiness to empty completely, to dose and to storage, as well as resealability and the optimal quantity of the product in the packaging (Williams et al., 2008).

In recent years, the environmental issues in the packaging area have been fought by using the reduction or changing in the material or following the recycling possibilities, unfortunately not focusing on the food loss. The new and added function of packaging is defined as the tool to reduce the food loss along the supply chain. In the best case, the “designer” of new packaging solutions

has to take in consideration the reduction of environmental packaging and food loss at same time, but in the majority of situations it could be necessary to increase the environmental impact link to packaging to reach a lower global environmental load due to the reduction of food loss.

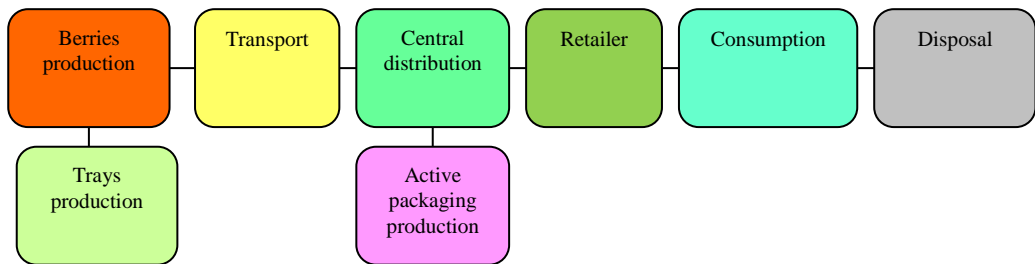
The aim of this section is to define objectively the role of packaging to reduce the food loss and thus the environmental load of each product-packaging solution through a specific model to determine the balance between the environmental impact of packaging and the environmental impact of the food losses due to changes in packaging.

The model used in this study was developed by Wikström and Williams in 2011 and can help to analyse packaging solutions with the porpoise of minimizing the environmental load of the packaging system. Using the LCA data from food supply chain and packaging production collected and analysed above in combination with food loss for the two different packaging solutions (active and traditional) it can be calculated the possible advantage of packaging solution to reduce global environmental load.

The model showed different adaption for this study, respect on the original one, due to the necessity to restrict the field of the investigation and the lack of information about the LCA evaluation of waste handling of food losses in the life cycle.

The boundaries of the LCA analysis was showed in the Figure 5.4 and represent the supply chain of raspberries, form agricultural step where the fruits were grew and harvested to the retailer without the consumer phase. As expressed above the Food waste management was not take into consideration, and the Fraction of food loss for each type of food packaging was calculated along supply chain and not in the consumption step as defined in the original model.

Figure 5.4. Boundaries of LCA analysis from berries production to packaging disposal.



The model used in this study is the following:

$$\frac{T2}{T1} < \frac{1 - L2}{1 - L1} + \frac{L1 - L2}{1 - L1} * \frac{F}{T1}$$

Where:

- T1: Environmental Impact of “traditional” packaging
- T2: Environmental Impact of “new” packaging in this study the active packaging solution in master bag
- L1: Fraction of Food Loss (“traditional” packaging)
- L2: Fraction of Food Loss (active packaging solution in master bag)
- F: Environmental Impact of food without packaging

The environmental load of packaging solution and food (raspberries) are expressed in kg of CO₂ equivalent taking into consideration as the functional unit in the LCA study the 2 sale units (250 g of raspberries).

The choice of this impact category was related to the need to provide an evaluation of the impact of the examined system in relation to climate change that can be readily communicated to and understood by consumer.

The fraction of the food loss was collected from literature for the “traditional” tray by WRAP report (2008, Figure 5.5). In the model was used the average value (20%). The fraction of food loss was assumed by interview on raspberries grower and retailer defining the possible reduction in the steps along supply chain (Figure 5.6). It was assumed that the reduction of loss was carried out in the field step due to the better management of the harvest, considering the possibility to storage the product for longer time coping the fluctuation in the consumer demand. At the retailer step, the increase of shelf life determine an food loss reduction due to the better management of the stockpile and the reference rotation on the shelves.

Figure 5.5. Food loss percentage along the raspberries supply chain in UK using the “traditional” packaging solution.



Figure 5.6. Food loss percentage along the raspberries supply chain in Italy using the “new” packaging solution (Active packaging solution).



The model used in this work are able to define the balance between the environmental impact of packaging and the environmental impact of the food losses due to changes in packaging. For this reason the increase in environmental impact from “new” packaging respect to the “traditional” must be below the environmental impact generated by the food loss reduction. In other words, the disequation is satisfied if the contribution of the food “saving” is higher than the larger environmental load of a new packaging solution.

The data came from the LCA analysis performed in the above section was showed in the Table 5.9. In the food value was estimated also the energy to refrigerate the products.

Table 5.9. Values inserted into the model to define the satisfaction of the disequation

Code	Definition	Value	Source
T1	Environmental Impact of “traditional” packaging	0.22 kg CO ₂ eq	LCA results
T2	Environmental Impact of “new” packaging (active atmosphere solution)	0.25 kg CO ₂ eq	LCA results
L1	Fraction of Food Loss (“traditional” packaging)	0.20	WRAP (2008)
L2	Fraction of Food Loss (active packaging solution in master bag);	0.10	Preliminary results
F	Environmental Impact of food without packaging	0.12 kg CO ₂ eq	LCA results

Using the data collected and shown in the Table 5.9 have been satisfied the disequation, determine the value of the ratio between T2 and T1 lower than the food loss saved in the right part of the disequation. The value came from the ratio of T2/T1 was 1.15 and the value of the food saved was 1.19.

In this study, the environmental load of the “new” packaging have an increase about 14% respect to the “traditional” one while the food loss saved correspond to the 10 %.

There is no doubt that the environmental impact can be significantly reduced if the food losses decrease, it is still unclear, however, to what extent new packaging can influence food losses directly or indirectly by influencing consumer behavior. It is not easy to separate these interacting factors.

One preliminary conclusion that can be drawn from these results follow the importance to study whether there is a risk that food losses increase when packaging design changes, for example, when the aim is less packaging material. The total environmental impact will most certainly increase if food losses increase, even if the impact from the packaging decrease. If the authorities want to reduce the total environmental load of the food packaging system, the next operation carried out to define a new legislation that take into consideration this approach.

5.4 CONCLUSION

The LCA evaluation was permitted to define which are the phases have an important role in the environmental load, the packaging system denoted an higher impact due to the collecting and the recycling processes, and the high quantity of packaging in comparison the fruit stored. It is clear that in this sector can be use different materials o different packaging solutions to reduce this load, for example reducing the weight of pack or following the biodegradable materials way that have a lesser impact (Razza et al., 2010).

The values of shelf life identified in this study came from the experimental evaluation and those values can able to define better the environmental sustainability of the systems due to the reduction of environmental impact correlate to the increase of shelf life value.

As a result of this study, it is possible to assess the environmental impact of the raspberries products stored in different packaging solution to identify the best solution to reduce the environmental burned. The LCA methodology was permitted to define as the best solution the raspberries packaged inside master bag with active devices.

Over the last years, the interest in the environmental impacts associated with food systems has strongly grown. Several studies have confirmed the relative importance of “food and beverages consumption” in contributing to environmental impacts (Bacenetti et al. 2015). Within the food chain, also the waste management processes contribute to the overall environmental burden of food products (FAO, 2013). Among the different mitigation strategies, several studies highlighted that the optimization of packaging solution can be an effective solution to decrease the environmental load of the food systems (Williams et al. 2011 and Piergiovanni, 2014). The future researches are need to define better the effect of the extension shelf life of this kind of product on the supply chain and the consumer habits. It is necessary improve the knowledge of the effect of the extension of shelf life on the sustainability in terms of environmental load, social and economic effect. One of goal of the FAO in the fight against the fame is the reduction of the global food waste as a contribution to feeding nine billion people by 2050. The limiting of this virtuoso process is the lack of knowledge of the causes and the reticence of the authorities and the politicians (Parfitt et al., 2010).

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6 - SHELF LIFE ESTIMATION OF STRAWBERRIES STORED IN TRADITIONAL AND PASSIVE MODIFIED ATMOSPHERE: A MULTIVARIATE APPROACH AS RELIABLE PROCEDURE

6.1 INTRODUCTION

Strawberry is a non-climacteric fruit and it must be harvested at full maturity to achieve the maximum quality in relation to flavour and colour (Peano et al., 2014). The fruits have short shelf-life due to physiological aspects such as high respiration rate, loss of firmness, mould susceptibility and breaking down tissues. In fact the most common reasons to complain by costumers are the expired, smashed and leaky fruits (Nunes et al., 2009) or, during the supply-chain, the *Botrytis* infection that is the major limiting factor in relation to quality of strawberries, potentially causing up to 50% loss (Hertog et al., 1999; Wszelaki and Mitcham, 2000). Many studies have been aimed to find the best food packaging in order to optimize the O₂ and CO₂ concentrations inside the packages, thus maintaining fruit and vegetable quality for a long period (Gomes et al., 2010).

To determine the efficacy of the packaging system, the most important aspect is the estimation of the shelf-life. This value can objectively define a gap between two or more packaging systems to extend the lifetime of product. To correctly estimate this value, it is necessary to follow a protocol to define the critical indicator that describes the most important relevant changes in the fruits during storage, and define its critical limit. The shelf life in this case is identified as the time at which the critical indicator reaches its critical limit.

Many reviews and books describe the methodologies applied to determine the shelf-life value but each author uses a specific grouping for the possible methodologies (Nicoli 2012; Hough 2010; Robertson 2009). Therefore the definition of which is the best methodology to determine the shelf life value seems “arbitrary“. In the fresh-fruits sector, the identification of the critical attribute and its limit can go through the simultaneous evaluation of different parameters due to the high variability of the products. In this study the estimation of the shelf-life has followed a multivariate approach as reported by Pedro and Ferreira (2006), taking into consideration different parameters at the same time. Once defined the shelf life of the traditional system, referred to macro-perforated PET tray with macro-perforated PET lid, the reached value was compared with shelf- life obtained storing strawberries in a passive atmosphere solution using a simple material (LDPE); a simple equipment was tested to offer a reliable packaging solution for small productions. In Italy in fact there are many small growers (Rava et al, 2002) which are exporting their products to various countries.

6.2 MATERIALS AND METHODS

6.2.1 Fruits: The strawberries were purchased in a macro-perforated PET tray with a PET rigid lid: this is the traditional sale unit containing 250 g of fruits. Strawberries (*Fragaria x Ananassa* Dutch.) cv. Asia from Northern Italy, picked at commercial ripening, were provided by a local supermarket in Milan and transported to the laboratory where they were immediately stored in a dark cold chamber (5±1 °C, 70 %RH) before packaging.

6.2.2 Packaging material: A macro-perforated PET tray with a PET rigid lid was used as “traditional” solution in this study.

The master bag (52*31 cm) used in the experimental plan was made from LDPE with the characteristics described in Table 6.1 including the gas permeability for oxygen transmission rate (O₂TR), carbon dioxide transmission rate (CO₂TR) and water vapour transmission rate (WVTR).

Table 6.1: Characteristic of plastic film

Material	Thickness (μm)	O ₂ TR ($\text{cc}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$)*	CO ₂ TR ($\text{cc}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$)*	WVTR ($\text{g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$)**
LDPE	25	4000	30000	21.7

* 23°C-0 % RH; ** 38°C-90 % RH.

6.2.3 Sensorial analysis:

Global acceptability: The consumers, after berries consumption, judged the global acceptability of berries indicating the positive opinion with “yes” or negative opinion with “no”. The global acceptability was expressed as the percentage of the positive acceptance judgment respect to the total consumers’ answer.

Sensory quality evaluation: A trained taste panel, with a minimum of six persons, evaluated the sensory quality of the packaged fruit. All sensory tests were performed in a room with artificial light, and sensorial properties such as bitterness, fermented taste, sweetness, acidity taste and visual calyx freshness were evaluated. A numerical score between 1 and 9 was given for each property to describe the sensory quality of the fruit. For negative quality bitterness, fermented taste, acidity taste scores 1–5 were associated with appreciated taste, whilst 6-9 scores were associated with off-taste or over-ripe. Score 6 represented the limit of these factors. For positive indicator as sweetness and visual calyx freshness, on the other hand, scores 6-9 represented a very good and fresh value, score 6 was still acceptable and scores 1-5 were associated with a non-acceptable taste or freshness. The cut-off score was fixed at 6 (Giménez et al. 2008).

Visual acceptability and Score acceptability: Fifty regular consumers of strawberries (nearly half male, half female) were recruited among students and employees of the University of Milan (Italy), between 21 and 60 years old. At each sampling time, the visual assessment of the acceptability was carried out in a 9-point hedonic scale (score acceptability). If the result was more than 6 points the berries were judged as acceptable, as established by Ares, et al., (2006) and Giménez et al. (2008). The visual acceptability was expressed as the percentage of the acceptance judgment respect to the total consumers answers.

6.2.4 Chemical and physical analyses

Maximum Force: The method used to assess product firmness intended to measure the structure resistance against the penetration. Firmness of strawberries was established using a dynamometer (Zwick Roell Instrumental Z10, Zwick GmbH & Co. KG, Ulm, Germany) through a single penetration test on each half berry on the equator of fruit (modified method from Gunnessa et al. 2009). Gunnessa et al. (2009) found that measurements taken on the equator (typically the highest point of the half fruit) gave more consistent results than from other positions.

The maximum force was assessed at least on 20 half strawberries per each sampling time. Each berry was positioned under the probe (3 mm diameter) and penetrated until 4 mm using a load cell of 10 kg (100 N), at test speed as 0.2 mm/s and with a trigger force as 5 g.

Percentage of rejected berries: Physically damaged and mouldy berries were visually counted at each sampling time and the results were expressed as percentage of rejected berries with respect to the total berries inside each tray. In particular, strawberry fruits showing surface mycelial development were considered decayed (Van deer Steen et al., 2002).

Colour: At each sampling time, colour of the berries was measured on 30 fruits taken from three different packages by an handheld Tristimulus colorimeter (Konica Minolta CR-300, Tokyo, Japan) determining L*, a* and b* parameters with a diameter 8 mm, 2° standard observer and a C as illuminant source. Before each measurement, the apparatus was calibrated on the Hunterlab color space system using a white ceramic tile (Minolta calibration plate, Y = 92.6, x = 0.3136, y = 0.3196). Colour was described as Hue angle (H°, expressed as $\arctg\ b^*/a^*$) and Chroma (C, expressed as $(a^{*2} + b^{*2})^{1/2}$) indexes.

Weight loss: The weight loss was determined gravimetrically by weighting each PET tray at time zero and during the storage using a Technical balance (MP-3000 Chyo Balance corp., Japan). Changes in fruit weight were expressed as percentage of weight loss.

Purée preparation: For biophysical and chemical analyses, the strawberries (100g) were puréed by handheld blender (Braun MR 4050 CA) for 30 s at high speed at each time of storage and kept frozen until the analyses were performed. The thawing was carried out in refrigerator overnight at 5 ± 1 °C .

Dry matter content: Determinations were made on 5g of fruit pulp by drying samples in oven set at 105°C. The samples were weighed after about 16 hours. The measurements were replicates three time. The results were expressed as g of dry matter for 100g of samples.

Titrateable Acidity (TA): After thawing of samples at 4°C overnight, TA was determined by titrating sample (2 g of homogenate + 40 mL of CO₂-free distilled water) with standardized 0.1 N NaOH to pH 8.2 (Phenolphthalein toning) by use of a pH meter (Basic 20+, Crison Instruments SA, Barcelona, Spain). TA was expressed as citric acid equivalents (grams of citric acid per 100 grams of berries)

Total soluble solid (TTS): After thawing at 4°C overnight, the berries pulp was put in the Automatic Refractometer model SMART-1 Atago®, (Atago CO.LTD, Tokyo, Japan). The results were expressed as BRIX°.

Respiration rate: Apparent respiration rate (RR) of the strawberries was measured at 5°C using the closed system method. The measurements were carried out in triplicate. Berries and the jars were equilibrated for 1 h at 5°C. Samples of about 100 g were then placed in open air in 0.5 L glass jars and tightly covered with metal caps equipped with silicone sampling ports. Headspace gas was periodically sampled (20-30 min) by means of a gas-tight syringe. Oxygen and carbon dioxide were detected and quantified by a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector and a steel column (2 m × 6 mm. CTR I Alltech, Milano), until the CO₂ level inside the jars reached 5%. Respiration rate was calculated from the linear regression of O₂ and CO₂ concentrations measured during the time of experiment and it was expressed as $ml \cdot kg^{-1} \cdot h^{-1}$.

$$RRO_2 = (\Delta[\%O_2] \cdot V) / ((\Delta t \cdot 100 + M))$$

$$RRCO_2 = (\Delta[\%CO_2] \cdot V) / ((\Delta t \cdot 100 + M))$$

Where:

V= Head space volume, ml

M= Mass of product, kg

t= Time, hour

6.2.5 Statistical analysis: Data were statistically evaluated by one-way ANOVA and multiple range test (Tukey method) to put in evidence significant differences among samples, using Statgraphics Plus v. 5.1 package (Statpoint Technologies, Inc. Warrenton, VA 20186, USA). The differences were considered significant at $P < 0.05$. In order to analyse the results from a multidimensional point of view, the quality parameters data were analyzed by Principal Component Analysis (PCA), using the Unscrambler v.9.7 software (CAMO, Norway).

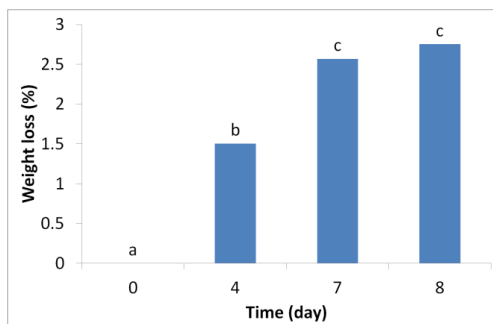
6.3 RESULTS AND DISCUSSION

6.3.1 Shelf- life definition of strawberries in PET macro-perforated tray (“traditional” solution)

The respiration rate was determined on the fresh fruits at 5°C and values obtained were $RRO_2 = 19$ ml (27.63 mg) $O_2 \cdot kg^{-1} h^{-1}$ and $RRCO_2 = 20$ ml (40 mg) $CO_2 \cdot kg^{-1} \cdot h^{-1}$; these values were in agreement with the values reported by Mitcham (2000; 20-50 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) but higher than values found in 3 different cultivars by Pelayo et al. (2003; 6-9 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$). The $RRCO_2 / RRO_2$ equal to 1.05 means that the fruits were in a good shape with an aerobically quotient. This value can be associated with an aerobically respiration due to the consumption of carbohydrates (Saltveit et al., 2014).

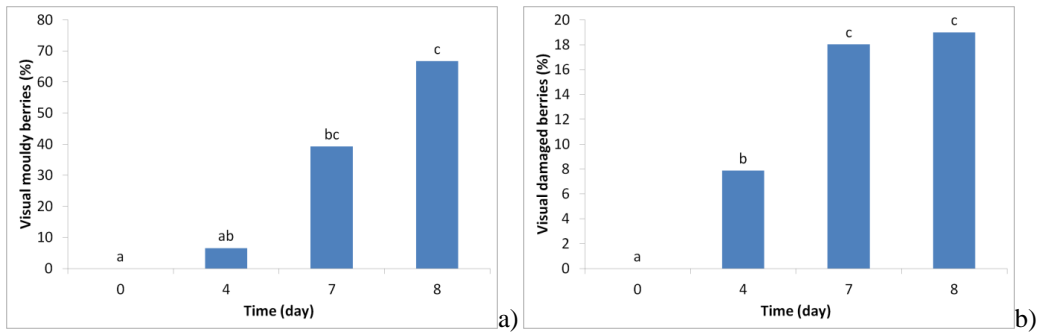
The water loss due to the transpiration involves a negative effect on the appearance of fruit, leading to shrivelling and a damaged on the surface. The limit of this loss for strawberries before marketability has been established to be approximately around 6% (Robinson et al., 1975). In this study the fruits lost around 3 % at the end of the storage (Figure 6.1); these values were lower than those found by Van der Steen et al. (2002; 5%) but the same loss was found in the strawberries held at 2 °C for 3 days + 4 days at 20 °C.

Figure 6.1. Weight loss of fruit during storage. Different letters show significant differences ($p < 0.05$).



The fungal infection on the surface tissue was visually assessed during storage. In this study, incidence of fungal growth gained high level after 4 days of storage, later the lag phase of fungi. The limit for this indicator is set at 5 % (Hertog et al., 1999), thus between 0 and 4 days the samples can be considered unacceptable (Figure 6.2). During storage the berries develop a leakage or damage on surface due to the weight loss, loss of firmness and enzymatic reactions. The limit for percentage of visual mouldy berry (10 % Sanz et al., 1999) has been reached between 4 and 7 days when the percentage of damaged berries arrived at 18 % at 7 days.

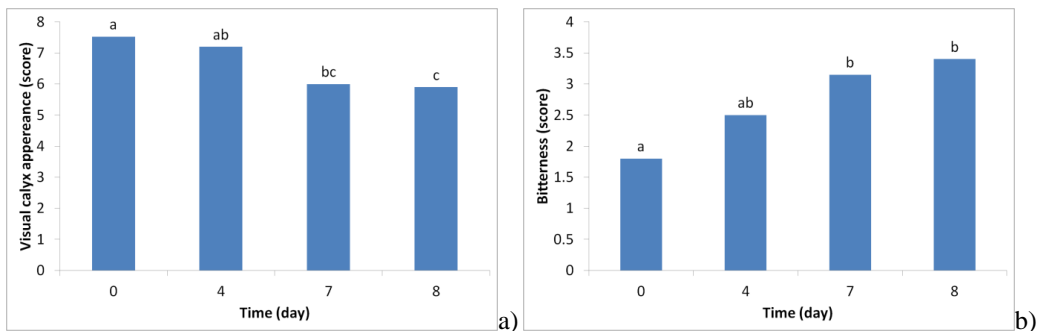
Figure 6.2. Visual rejected berries during storage, a) moulded, b) damaged. Different letters indicate significant differences ($p < 0.05$).

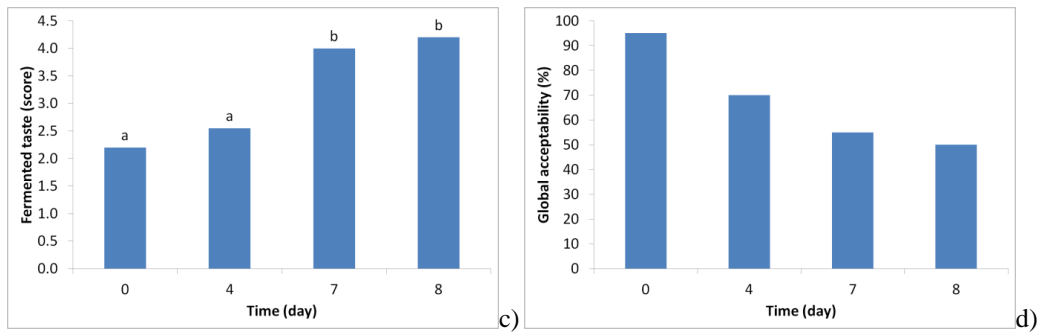


One of the most important factor that defines the changes in the strawberries quality is the sensorial analyses. Sensory quality is related to the characteristics of the food and how consumers perceive them (Costell, 2002). With increasing of the storage time, deterioration starts with decay, fermentation and bruising (Ares et al., 2009). Figure 6.3 a-d summarizes the results of the sensory attributes. Panelists detected two main off-flavours: the bitterness and the fermented taste; and one visual quality index refers to the browning of the sepals in the calyx. This last indicator defines the freshness of the fruits due to the browning of the sepals that change their colour from green to yellow-brown.

The development of sensory defects contributed to reduce the global acceptability of fruits as showed in the Figure 6.3-d.

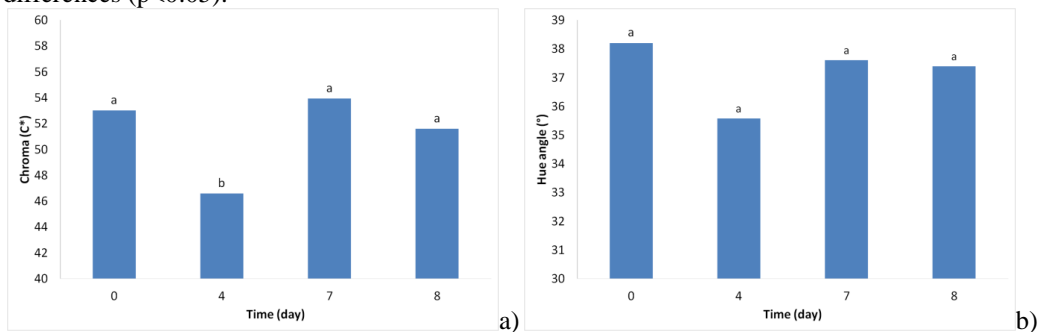
Figure 6.3 a-d. Sensory attribute (Visual calyx appearance, bitterness, fermented and global acceptability). Different letters indicate significant differences ($p < 0.05$).





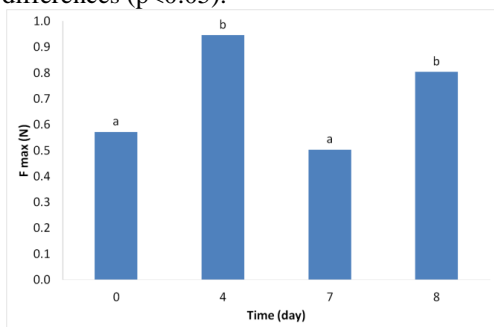
The changes in colour parameters during storage were cultivar dependent as reported by Pelayo et al., (2003). In this study any evolution was defined, the colour saturation (Chroma) and the Hue angle didn't change during storage as showed in Figure 6.4. This behaviour could be correlated with the conservation of the anthocyanins and the pH of the fruits that maintain the starting values as found in different works (Sanz et al., 1999 and the Pelayo et al., 2003).

Figure 6.4 a-b. Colour evaluation (chroma and hue). Different letters indicate significant differences ($p < 0.05$).



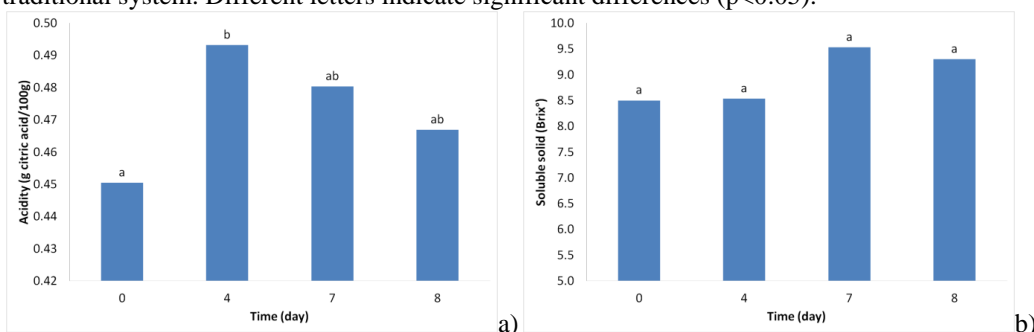
The maximum force is a very important indicator of strawberries' quality and it can be used to determine the texture changes. As reported for other quality indicators the firmness changes can be correlated with the cultivar used in the experiment; in fact, as reported by Pelayo et al., (2003) the firmness, in terms of the maximum force of penetration, changed during storage in different ways in relation to various cultivars used in the experiment. In this study there was a significant variation during storage that could be correlated with the high intrinsic sample variability (Figure 6.5).

Figure 6.5. Firmness change during storage of strawberries. Different letters indicate significant differences ($p < 0.05$).



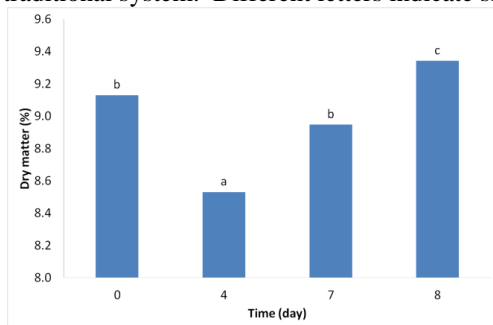
For an acceptable taste, a maximum 0.8% Titratable Acidity (TA) and/or a minimum 7% total soluble solid (TSS) have been recommended (Mitcham et al., 1996). In this study the strawberries fitted within the recommended TA value and the TSS. The acidity decreased after 4 days and reached a value similar that found at beginning, while the TSS showed a different behaviour, maintaining the same value for the entire experimentation, in fact not significant difference was found (Figure 6.6).

Figure 6.6. Changes in titratable acidity and soluble solid content in strawberries stored in traditional system. Different letters indicate significant differences ($p < 0.05$).



The changes in the dry matter was ascribed to the product variability and continuous senescence of fruits that loss water (transpiration) and solid (catabolism). The values obtained in this study (Figure 6.7) were in the range between 7.4-10.8 as also described by Skrede et al. (2011) for strawberry fruits.

Figure 6.7. Changes in titratable acidity and soluble solid content in strawberries stored in traditional system. Different letters indicate significant differences ($p < 0.05$).



6.3.1.1 Definition of shelf life by Multivariate analysis

Multivariate analysis (MVA) is based on the analysis of more than one statistical outcome variable at a time. This technique can be used to assess the shelf life value considering many different parameters and taking into account the effects of all variables on the responses of interest.

One of the most useful techniques based on this approach is the Principal Component Analysis (PCA), which aims at finding a new set of axes in multivariate space that better describes the structure in the data. These new axes are called Principal Components (PC) and are built by linear combinations of the original variables (Malinowski., 1991; Wold et al., 1987).

Following the steps defined by Pedro and Ferreira (2006) study the multivariate approach was applied.

Firstly a matrix was carried out collecting in the columns the results of physical and sensorial analyses while rows represent the time at which the results were obtained. In this matrix 3 replicates of the raspberries analysis performed along 2 years for different batches of samples were taken into account. This structure of the columns is necessary in order to keep samples spread in a single multivariate space which would reveal time dependence in the PCA; the variables present different scales, and the auto-scale procedure was performed to obtain to obtain the X_a matrix. The columns of X_a have means equal to zero and unit variance (Eq. 6.1):

$$X_a = \frac{X_{n,k} - \bar{X}_k}{S_k} \tag{6.1}$$

where X_k and S_k are, respectively, the mean and the standard deviation of the elements of the k -th column of X and X_a , k and $X_{n,k}$ are typical elements of X_a and X . n is the number of points in time where evaluations were conducted.

Secondly build up shelf-life charts (PC scores vs. time) for the first R PC and identify the scores which are time-related and for each of the time-related PC, identify their reaction order and determine the kinetic parameters using the PC scores as properties.

Thirdly to identify gathering the cut-off criteria for the score time-related PC using the loading values of the PCA should be used the following procedure (Eq. 6.2):

- 1) Place the reference values for each property into the x vector and pre-process it using the parameters determined in equation 3.5 to obtain X_a .
- 2) Use the loadings matrix to calculate the cut-off criteria that is the maximum acceptable scores for each time-related PC:

$$T_{crit} = X_a * L_m \tag{6.2}$$

where X_a is the row vector of reference values and L_m is the loadings matrix of the time-related PC for storage condition (Pedro and Ferreira 2006).

The changes in raspberries quality indices stored in traditional packaging can be described by using few quality indices with respect to all the parameters tested in this study. The analysis of loading of the original matrix established that the loading that have an impact more than 0.3 was collected to use it in the final matrix and to estimate the global quality indices. This selection was carried out to determine the best quality indices that describe the changes in the berries during storage

A total of 3 PCs were retained from the PCA applied to the ratings of the 6 attributes used to describe the quality attributes of strawberries. Visual mouldy berries, visual damaged berries, weight loss, bitterness and fermented taste and global acceptability.

The first of the two principal components explain 88% of variability. A separation of the samples according to the storage conditions is shown on the scores plot (Figure 6.8), where the number beside each point represents the storage time in days. In particular, samples were distributed along PC1 according to the storage time. Loadings revealed the weight of attributes responsible for product degradation (Figure 6.9) and the selected components represent the most important factor in the quality decay identification. It can be seen that those variables which increase in time have positive PC1 loadings whilst those which decrease present negative values. All parameters studied in this analysis have shown a positive values defining an increase trend during berries storage except the global acceptability that have shown a negative effect.

As expected, the samples closed to initial value of the study were characterized by high value of global acceptability whereas the sample at the end of the storage were characterized by high values of the other quality parameters (visual mould and damaged berries, weight loss and bitterness fermented taste) due to the senescent. These considerations are in agreement with the changes of quality indices reported above.

Figure 6.8. Scores plot of Principal Component

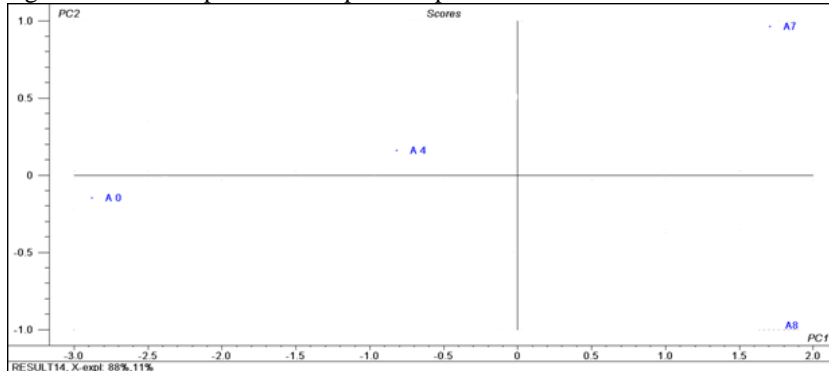


Figure 6.9. Loadings plot of Principal Component

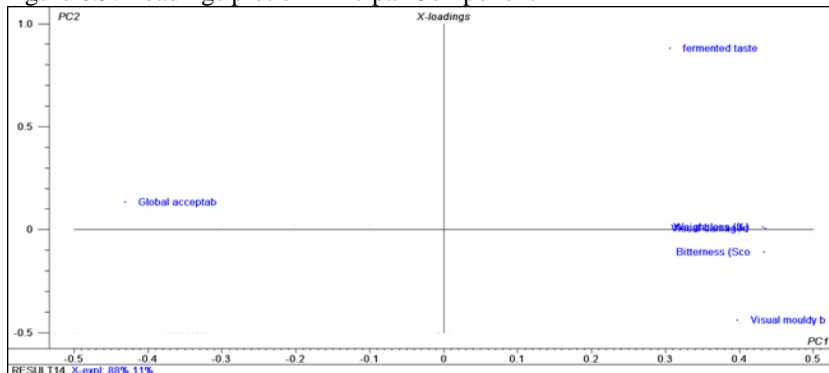


Table 6.2: The matrix of quality indices used in the PCA to identify the best parameters to estimate the shelf life value

Time (day)	Weight loss (%)	Visual mouldy berries (%)	Visual damaged berries (%)	Chroma (C*)	Hue angle (°)	Maximum Force (N)
0	0.00	0.00	0.00	53.02	38.21	0.57
4	1.50	6.59	7.87	46.60	35.58	0.95
7	2.57	39.24	18.03	53.94	37.60	0.50
8	2.75	66.71	19.00	51.60	37.39	0.80

Time (day)	Firmness evaluation (Score)	Sweetness (Score)	Bitterness (Score)	Fermented Evaluation (Score)	Global acceptability (%)
0	1.76	2.78	1.00	1.22	70.00
4	2.31	3.08	1.39	1.42	70.00
7	2.08	2.25	1.75	1.89	50.00
8	2.33	2.14	1.89	1.42	40.00

The multivariate approach is able to consider the main quality parameters that have effect on the quality changes in strawberries. Using the property of time-structure of PC1 in function of time, the Figure 6.8 was defined. Given that the overall degradation reaction followed pseudo-zero-order kinetics for berries stored in this packaging solution, the linear equation with parameters was reported in Table 6.3.

Table 6.3. Linear equation parameters and fitting of pseudo-zero-order kinetic

a	b	R ²
0.6349	-3.0157	0.989

The limits used for estimating the PC critic for passive packaging solution was reported in Table 6.4. Using 6 parameters simultaneously the efficacy of the critical indicator is improved in

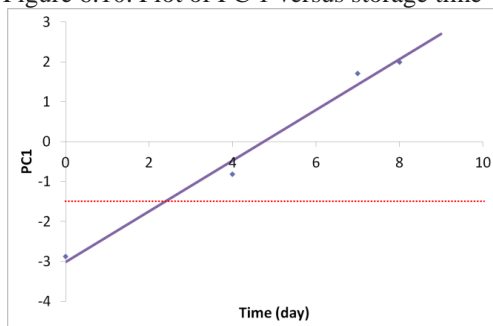
comparison with the classical protocol that identify only one parameter at time. For this kind of products is necessary to use more than one quality indicator to estimate the shelf life value due to the high intrinsic variability of samples.

Table 6.4. Limit values for different quality parameters to identify the critic PC for passive packaging solution

Variable	Unit	Limit	References
Visual mouldy berries	%	5	Hertog et al., 1999
Visual damaged berries	%	10	Sanz et al., 1999
Weight loss	%	6	Robinson et al. 1975
Bitterness	Score	6	Jouquand et al., 2008
Fermented taste	Score	6	Jouquand et al., 2008
Global acceptability	%	50	Lareo et al., 2009

Applying the reference values for each quality attribute expressed above and using the equation (6.2), a critical PC1 score value of 1.68 was obtained (refer to Figure 6.10), which corresponds to a shelf life value about 2.2 ± 0.5 days. This value is lower than that identified by Mitcham (2000; 7 days) and Pelayo et al. (2003) that found for different cultivars values ranging from 5 to 7 days. The shelf life value depends on different factors and, among others, the cultivar and the disease pressure (Pelayo et al. 2003).

Figure 6.10. Plot of PC 1 versus storage time



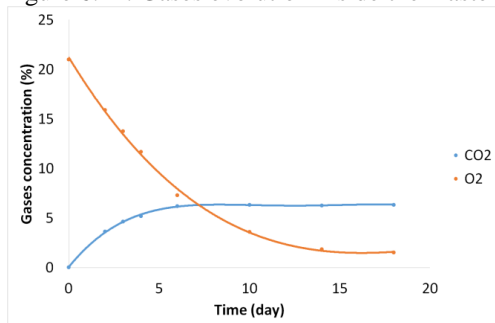
6.3.2 Definition of shelf life for Passive packaging solution

The modified atmosphere packaging (MAP) and the low storage temperature are some of the most used methods to preserve quality and safety of fruit and vegetable products (Bogaert et al., 2004; Sandhya, 2009). Passive modified solution consists in an atmosphere modification thank to the relationship between product respiration and film permeability during storage (Mangaraj et al., 2009). Inside packages, O₂ is used by the fruits in respiration metabolism to produce CO₂. The closed condition due to the film permeability allows lower concentration of oxygen and higher concentration of CO₂ to be reached, causing a reduction in product’s respiration rate and a consequent slowing down of senescence and decay phenomena (Das et al., 2006). Unfortunately reaching and maintaining the optimal gases conditions is a slow process that allows alteration in the fruits before selling.

In order to extend the shelf life and maintain the quality, the MAP solution requires careful design. In fact, an excessive O₂ depletion carried out under anaerobic conditions and CO₂ accumulation (toxic condition), for example, allows a fast quality degradation.

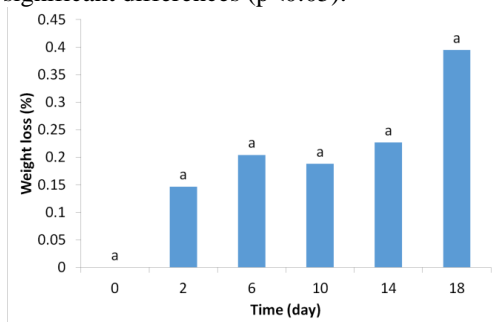
The aim of this study was to define the shelf life of strawberries stored in passive modified atmosphere following the most important quality indices during storage in a master bag solution. The effect of the film used to package the strawberries on the evolution in the headspace gases composition is shown in the Figure 6.11. The changes in gases concentration inside the master bag moved differently from the normal atmosphere composition (21% O₂ and 0.03% CO₂). The oxygen value went down reaching the danger limit for the strawberries (2%, Kader, 1997) after 10 days, across the optimal limit (10% Van der Steen et al., 2002). The carbon dioxide level of 5 % was reached at 5 days and kept until the end of storage. This value was not enough to reduce the growth of moulds and yeasts for longer time and reduce the fruit respiration. In fact the recommended level for carbon dioxide is in the range from 10 to 15 % (Van der Steen et al., 2002; Almenar, 2005 and Joles et al., 1994).

Figure 6.11. Gases evolution inside the master bag with strawberries



The water loss involves a negative effect on the appearance of strawberries leading to shrivelling and a dull-looking berry surface. The maximum limit for strawberries before marketability has been reported to be approximately 6% (Robinson et al., 1975). During storage, the weight loss didn't reach the limit and, as expected, didn't show any significant differences thanks to the plastic film that maintained the high humidity level inside the master bag, delaying the fruits dehydration as reported in Figure 6.12. The value recorded at the end of the storage was in the same range found in other works (Peano et al. 2014; Nielsen and Leufve'n, 2008).

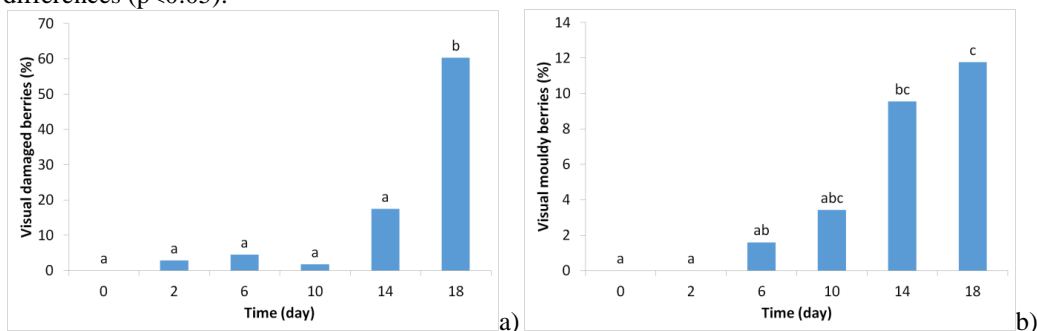
Figure 6.12. Weight loss of strawberries changes during storage. Different letters indicate significant differences (p<0.05).



A berry was considered damaged (unacceptable) if it was visibly affected by any form of deterioration, visible fungal growth not included, on at least 1/3 of the surface (Sanz et al., 1999) and the limit of the acceptance was set at 10% referred to a package. While the visual fungal

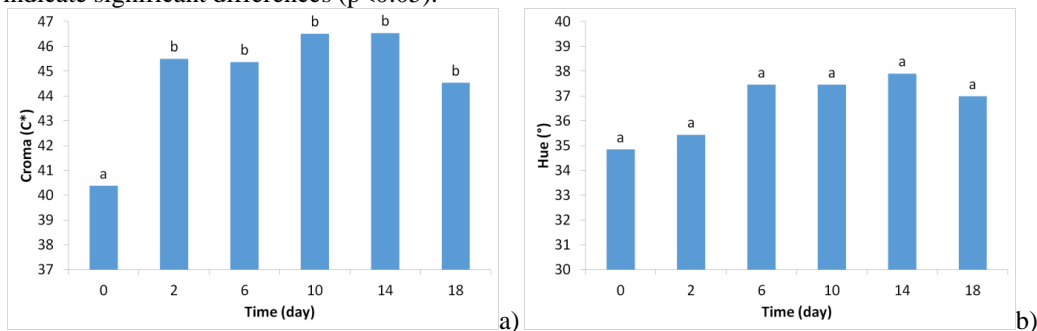
growth, in most of the case by *Botrytis*, was observed in the study and its limit was set at 5% Hertog et al., 1999). In this study the damaged berries exceed the limit between 10 to 14 days and reached an high level of damaged fruits at the end of the storage (60%, Figure 6.13a); these changes can be correlated with the low oxygen concentration causing the switch the fruit metabolism from aerobic to anaerobic (Beaudry, 2000). The incidence of visual mould on berries was detected since the 6th day and exceed the limit between 10 to 14 days of storage. The head space gases concentration during storage allowed the delay of mould growth. These condition, unfortunately, haven't blocked the mould growth recording a value at the end of the storage around 12 % (Figure 6.13b).

Figure 6.13. Changes in visual unacceptable berries during storage in passive solution, a) represents the visual damaged berries b) the visual mouldy berries. Different letters indicate significant differences ($p < 0.05$).



The most important factor for consumers to choose the fruit is colour (Del-Valle et al., 2005). The changes in colour parameters during storage are cultivar dependent as reported by Pelayo et al., (2003). In this study and for this strawberry cultivar the Hue didn't show any changes during storage as showed in Figure 6.14 while the Chroma showed an increase from time 0 to day 2 due to the quickly changing in the headspace of CO₂ concentration. This behaviour could be correlated with the slight variation of the intracellular pH of the fruits allowing to modification of the colour from anthocyanins (Sanz et al., 1999 and the Pelayo et al., 2003).

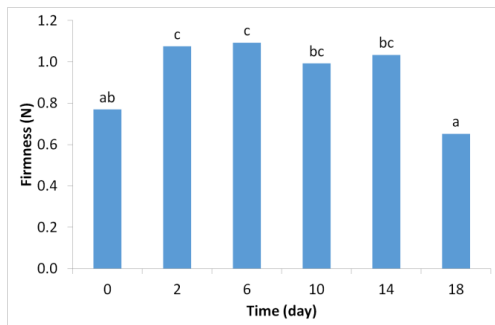
Figure 6.14. Colour changes for two colour indices chroma (a) and hue (b). Different letters indicate significant differences ($p < 0.05$).



Fruit firmness is defined as the ability of fruit to maintain integrity, shape and avoid the release of juices. Consumers are able to assess fruit texture through a simple visual evaluation before

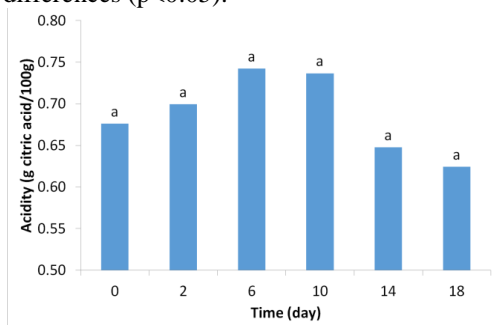
purchasing; if the product does not meet their requirements in term of firmness and colour, the consumers reject it. The firmness of fruits stored in the passive modified atmosphere recorded an slightly increase after only 2 days, probably due to the effect of the CO₂ concentration, being this gas involved in increasing or maintaining the firmness of fruits (Larsen and Watkins, 1995). Usually, this increase happens by higher carbon dioxide concentration (15-20%) but in this study the effect on the fruits seems proportional to the CO₂ concentration. In fact the mechanism by which both low temperature and CO₂ affect strawberries firmness is not yet understood. A possible explanation of this phenomena regards an indirect effect of CO₂ on the apoplastic pH with the consequent precipitation of soluble pectins and the improvement of cell-to-cell bonding (Harker et al., 2000). Following this theory the proportional effect of the CO₂ concentration might explain the firming response observed in this study. After 6 days until the end of the storage the berries become soft due to the senescence of the fruit carried out from the enzymes presented inside the cells, reaching limit for this indicator set at 1.05 N (Hietaranta and Linna 1999) (Figure 6.15).

Figure 6.15. Firmness changes of strawberries during storage. Different letters indicate significant differences (p<0.05).



The total titratable acidity (TTA) calculated as citric acid, which is the dominant acid in strawberries. In this study the TTA have been shown any significant differences and did not exceed too much the optimal condition to sell the strawberries, fixed at 0.7 % (Kader 1999; Figure 6.16).

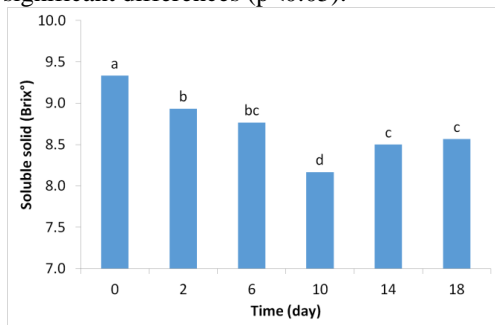
Figure 6.16. Acidity changes of strawberries during storage. Different letters indicate significant differences (p<0.05).



Total soluble solids is a critical factor for determining fruit quality and consumer acceptability (Kafkas et al., 2007). Sugars are the main soluble metabolites, and include glucose, fructose and sucrose comprising the 99% of the total sugar content (Kafkas et al., 2007). The TTS significantly

decreased throughout storage reaching a value around 8.5° and never exceeded the consumer acceptability limit fixed at 7° (Kader, 1999; Figure 6.17). This reduction could be explained by the hydrolysis of sugar and utilization of the corresponding reducing sugars in fruit respiration; data were in agreement with some results obtained for strawberries stored in MAP (Almenar et al., 2007 and Garcia et al., 1998).

Figure 6.17. Soluble solid changes of strawberries during storage. Different letters indicate significant differences ($p < 0.05$).

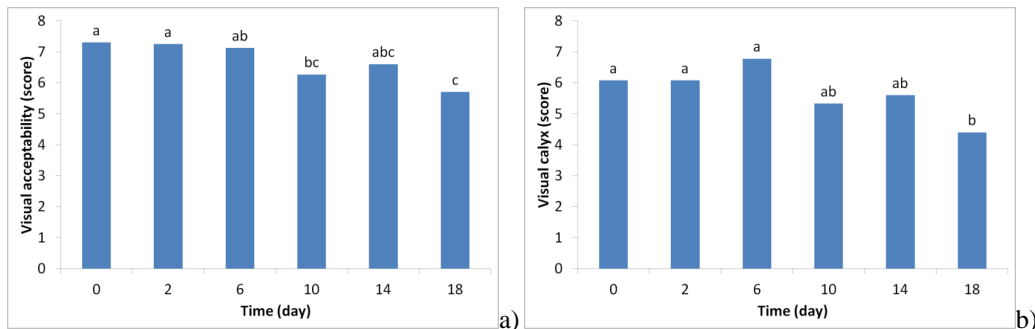


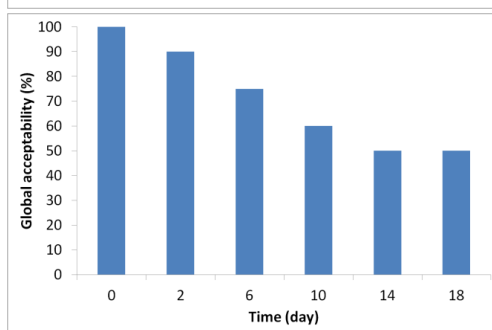
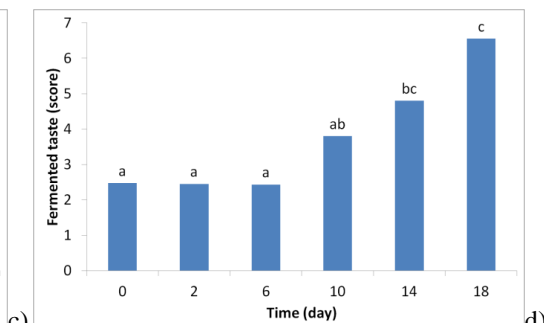
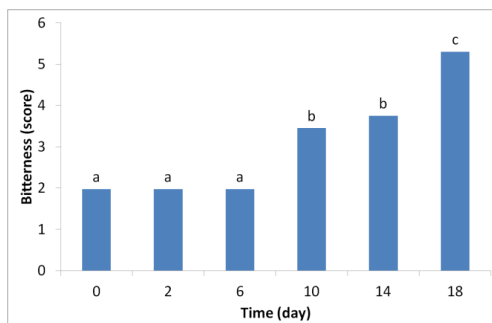
One of the most important factor that defines the changes in the strawberries quality is the sensorial analysis. Sensory quality is related to the characteristics of the food and how consumers perceive them (Costell, 2002). Figure 6.18a-e summarizes the results of sensory attributes.

Panelists detected two main off-flavours: the bitterness and the fermented taste; and one visual quality index refers to the browning of the sepals in the calyx. This last indicator defines the freshness of the fruits due to the browning of the sepals that change their colour from green to yellow-brown. Acidity taste and Firmness taste didn't show any significant evolution (data not shown).

The off-taste indices reached the limit (6 score) around 18 day of storage (Figure 6.18c-d). The acceleration of the production in off-taste happened after 6 days of storage when the fruit reached the limit of the tolerance for oxygen concentration lead to the anoxic condition for the fruit. Also the global acceptability reached its limit after 10 days of storage. The visual calyx freshness (Figure 6.18a-b) showed a decrease of the green appearance of the sepals after 6 days of storage, defining this period as the common point where the sensorial indicators record the change in the evaluations.

Figure 6.18a-e. Sensory attribute (Visual acceptability, Visual calyx, Bitterness, Fermented taste and Global acceptability). Different letters indicate significant differences ($p < 0.05$).





c) d) e)

To define the shelf life value, also for passive packaging solution the multivariate approach as described from Pedro and Ferreira (2006) was used. The proposed approach aims to combine the limit of several quality indices to define only one critical indicator to estimate the time at which the samples don't satisfy the quality requirements.

The changes in strawberries quality indices stored in passive packaging solution can be described by using few quality indices with respect to all the parameters tested in this study. The analysis of loading of the original matrix established that the loading that have an impact more than 0.3 was collected to use it in the final matrix and to estimate the global quality indices. This selection was carried out to determine the best quality indexes that describe the changes in the berries during storage. In fact 10 indices were used to evaluate the shelf life of berries using PCA approach respect to all parameters measured in this work as reported in Table 6.5. The selection defined the visual acceptability, bitterness, fermented taste, global acceptability, maximum force, visual damaged and mouldy berries, weight loss and oxygen concentration as the main quality indices to describe the quality decay.

Table 6.5. Quality indices recorded for strawberries

Time	Acceptability (score)	Visual calyx (score)	Taste texture (score)	Sweetness (score)	Bitterness (score)	Taste Acidity (score)	Fermented taste (score)	Global acceptability (%)
0	7.10	6.1	5.0	7.0	2.0	3.0	2.5	100
2	7.00	6.1	4.8	6.2	2.0	3.3	2.5	90
6	6.83	6.8	5.7	4.4	2.0	3.9	2.4	75
10	6.03	5.3	6.0	4.3	3.5	3.8	3.8	60
14	5.90	5.6	4.6	4.0	3.8	3.0	4.8	50
18	5.85	4.4	5.4	5.3	5.3	5.1	6.6	50

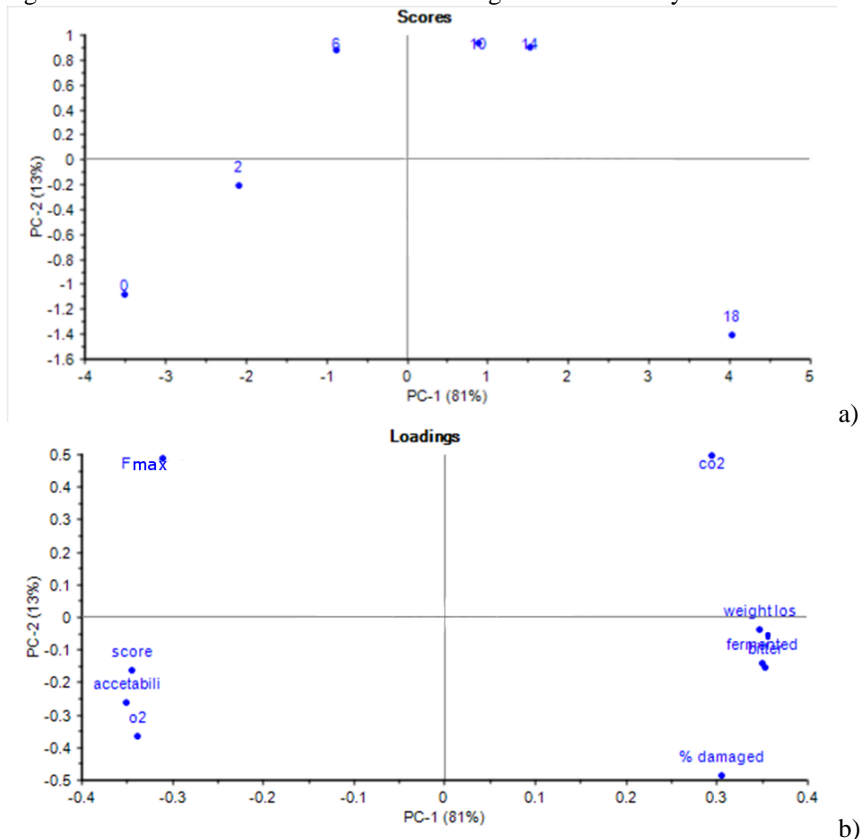
Time	TTS (BRIX°)	TA (%)	Max Force (N)	Croma (C*)	Hue (°)	Visual mouldy berries (%)	Visual damaged berries (%)	Weight loss (%)	O₂ (%)	CO₂ (%)
0	9.33	0.68	1.10	40.38	56.00	0.00	0.00	0.00	21.00	0.03
2	8.93	0.70	1.08	45.50	51.50	0.00	2.85	0.15	15.91	3.65
6	8.77	0.74	1.09	45.37	56.11	1.59	4.49	0.20	7.31	6.21
10	8.17	0.74	0.99	46.51	56.85	3.42	5.18	0.19	3.61	6.33
14	9.27	0.65	1.03	46.54	59.54	9.55	17.48	0.23	1.86	6.26
18	8.57	0.62	0.65	44.53	57.64	11.76	60.27	0.39	1.53	6.35

The first of the two principal components explain 81% of total variability. A separation of the samples according to the storage conditions is shown on the scores plot (Figure 6.19a), where the number beside each point represents the storage time in days. In particular, samples were distributed along PC1 according to the storage time. Loadings revealed the weight of attributes responsible for product degradation (Figure 6.19b).

The samples at beginning of the study were characterized by high value of oxygen concentration and global acceptability. During the storage, the samples showed an increase of the negative indices, in fact the samples after 18 days were characterized by high value of visual damaged berries, and off-flavour (bitterness and fermented taste).

The loadings plot in Figure 6.19b reveals the key attributes responsible for product degradation. It can be seen that those variables which increase in time have positive PC1 loadings whilst those which decrease present negative values. The Texture, the Visual acceptability and Score and oxygen concentration have shown a negative values defining a decrease trend during storage, while Weight loss, Fermented and Bitterness taste, Carbon dioxide concentration and Rejected berries have shown a positive values defining an increase trend during berries storage.

Figure 6.19a-b. Plot of the Scores and Loadings of the PC analysis



The selected quality parameters permitted to evaluate the best indicators to estimate the shelf life value.

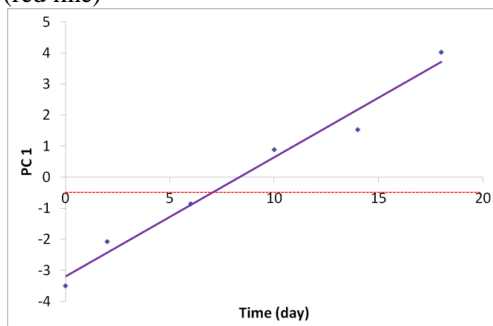
The multivariate approach can be able to use all parameters that have effect on the quality changes in strawberries. The overall degradation reaction followed pseudo-zero-order kinetics for samples and define the kinetics of degradation (Figure 6.20).

Applying the reference values reported in Table 6.5 for each quality attribute expressed above and using the equation (6.2), a critical PC1 score value of -0.61 was obtained (refer to Figure 20), which corresponds to a 6.6 ± 0.5 days of shelf life. In other words, the passive solution can extend the shelf life of berries, from about 2 days to about 6 days.

Table 6.6. Limits used to calculate the PC critic in the multivariate analysis.

Quality indicator	Unit	Limit	References
Visual acceptability	Score	6	Giménez et al., 2007
Bitterness	Score	6	Jouquand et al., 2008
Fermented Taste	Score	6	Jouquand et al., 2008
Global Acceptability	%	50	Piagentini et al., 2005
Maximum force	N	1.05	Hietaranta and Linna 1999
Visual damaged Berries	%	10	Hertog et al., 1999
Visual mouldy Berries	%	5	Sanz et al., 1999
Weight loss	%	6	Robinson et al., 1975
O ₂ concentration	%	2	Beaudry, 2000
CO ₂ concentration	%	25	Kader, 1997

Figure 6.20. The interpolation of the pseudo-zero order kinetic for PC 1 value with the critic PC (red line)



6.4 Conclusion

The use of Principal Component Analysis is a useful technique to combine different quality indices into only one simplifying the decision making process by the definition of a single cut-off criterion. This is advantageous to define and compare the shelf life values of fruits stored in different conditions, in terms of environmental, packaging or cultivar. Using more than one quality indicators the variation of fruits can be evaluated using this technique without exploring all parameters that determine the quality value.

The strawberries stored in “traditional” solution had a shorter shelf life, as defined in this study, but passive and active atmosphere solutions could allow the shelf life extension (Peano et al., 2014, Hertog et al., 1999 and Aday et al., 2011). In the second part of this study the passive solution has

been investigated evaluating the effectiveness of the packaging film in maintaining the quality attributes of fresh strawberries. The passive packaging solution can extend the shelf life of strawberries with respect to the “traditional” solution. The master bag, in addition, can contribute to better manage the fruit in the warehouse, at grower, at distribution center or at retailer point.

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7-ACTIVE PACKAGING IN MASTER BAG SOLUTIONS OF STRAWBERRIES

7.1 INTRODUCTION

Nowadays the consumer requests better fresh-like products with longer shelf life (Guynot et al., 2003). To reach this goal, the food packaging technology should be improved. The use of modified atmosphere protection and active packaging for fresh fruits and vegetables is of particular interest. The combination of storage at low temperature and modified atmosphere in pack with different levels of oxygen and carbon dioxide allows the achievement of the shelf life extension of fresh products maintaining the quality traits.

The proper concentrations of oxygen and carbon dioxide range from 5 kPa to 10 kPa for O₂ (Joles et al. 1994) and 15-20 kPa for CO₂ (Beaudry, 1999) and they can be reached through the use of oxygen absorbers and carbon dioxide emitters with a suitable film in terms of oxygen permeability. Lower levels of oxygen can reduce the respiration and decay (Beaudry, 1999), moreover high concentrations of CO₂ can contribute to reduce fungal growth (Brown, 1992), loss of firmness (Jacxsens et al., 2000; Day, 2001) and, in lower proportion, reduce the respiration rate. Under or over, respectively, the concentration of those gases can promote the off-flavour production (e.g. acetaldehydes, ethanol and ethyl acetate) and fruit injury (Pesis, 2005). This phenomena can limit the use of this techniques.

The optimization of an active packaging solution for fruits and vegetables, based on the insertion of oxygen absorbers and carbon dioxide emitters into master bags, requires the knowledge of the responses in terms of quality evolution but it is also dependent on the primary packaging features, like gas permeability, volume, quantity of food product etc.

In this part of the work, the Response Surface Methodologies (RSM) have been applied in order to define which factors, together with their interactions, can really contribute to reach the optimal conditions in terms of gases concentration inside the master bag. RSM is a collection of mathematical and statistical procedures based on the fitting of a polynomial equation to the experimental data describing the behavior of a data set with the objective of making statistical previsions. The goal is to simultaneously optimize the values of some factors to realize the best system performance (Bezzerra et al., 2008). In our study, the number of carbon dioxide emitters and oxygen absorbers, the unfilled volume of the master bag and the storage time were selected as factors to be optimized in order to extend the shelf life of strawberries.

7.2 MATERIALS

Fruits: Fresh strawberries were purchased in a macro-perforated PET tray with a PET rigid lid, containing 250 g of fruit (traditional packaging). Along the supply chain, the trays were transported into cardboard crates (40x30x9 cm) in which eight trays fit. Strawberries (*Fragaria x Ananassa* Dutch.) cv. Asia from Northern Italy picked at a commercial ripening were provided by a local supermarket and transported to the laboratory where they were immediately stored in a dark cold chamber (5±1 °C, 70 %RH) before packaging.

Packaging material: The master bag (52*31 cm) used in experimental plan was made from low density polyethylene (LDPE) with the characteristics (Table 7.1) including the gas permeability for oxygen transmission rate (O₂TR), carbon dioxide transmission rate (CO₂TR) and water vapour transmission rate (WVTR).

Table 7.1: Characteristic of plastic film

Material	Thickness (μm)	O ₂ TR ($\text{ccm}^{-2}\text{day}^{-1}$)*	CO ₂ TR ($\text{ccm}^{-2}\text{day}^{-1}$)*	WVTR ($\text{gm}^{-2}\text{day}^{-1}$)**
LDPE	25	4000	30000	21.7

* 23°C-0 % RH; ** 38°C-90 % RH.

The trays were inserted in the master bag which was closed with a packaging machine CVP System, Inc. (Downers Grove, Illinois, USA) filling the master bag with air gas through snorkels, which are retracted from the pouch prior to sealing. The atmosphere inside the master bag was evacuated in order to prevent the crash of fruit trays, filled with gas air and sealed. The air inserted into master bags created a pillow pouch that never collapsed into the trays during the storage. The gas volume inside each bag was approximately 9500 c m³. To reach the optimal relative humidity (about 90%) a blotting paper with 6 ml of de-ionized water was placed inside the master bag. The fruits were stored in a dark cold chamber at 5±1 °C, 70 %RH and their quality was monitored at the times defined by the design of experiment (Tables 7.2 and 7.3).

7.3 METHODS

Score acceptability & Visual acceptability: Fifty regular consumers of strawberries (nearly half male, half female) were recruited among students and employees of the University of Milan (Italy), between 21 and 60 years old. At each sampling time, the visual assessment of the acceptability was carried out in a 9-point hedonic scale (defining the score acceptability). If the result is more than 6 points the berries are judged as acceptable, as established by Ares, et al. (2006) and Giménez et al. (2007). The visual acceptability was expressed as the percentage of the acceptance judgment respect to the total consumers' answers.

Global acceptability: The consumers, after berries consumption, judged the global acceptability of berries indicating the positive opinion writing “yes” or negative opinion indicating “no”. The global acceptability was expressed as the percentage of the positive acceptance judgment respect to the total consumers' answers.

Maximum Force: The method used to assess the product firmness intended to measure the structure resistance against the penetration. strawberries Firmness was established using a dynamometer (Zwick Roell Instrumental Z010, Zwick GmbH & Co. KG, Ulm, Germany) through a single penetration test on each half berry on the fruit equator (modified method from Gunnessa at al. 2009). Gunnessa at al. (2009) found that the measurements taken on the equator (typically the highest point of the half fruit) gave more consistent results than in other positions. The maximum force was assessed at least on 20 half strawberries per each sampling time. Each berry was positioned under the probe (3 mm diameter) and penetrated up to 4 mm using a load cell of 10 kg (100 N), at test speed of 0.2 mm/s and with a trigger force of 5 g.

Percentage of rejected berries: Physically damaged and mouldy berries were visually counted at each sampling time and the results were expressed as the percentage of rejected berries respect to the total berries inside each tray. In particular, strawberry fruits showing surface mycelial development were considered decayed (Van deer Steen at al., 2002).

Colour: At each sampling time, the berries colour was measured on 30 fruits taken from three different packages through a handheld Tristimulus colorimeter (Konica Minolta CR-300, Tokyo,

Japan) determining L*, a* and b* parameters with a diameter 8 mm, 2° standard observer and a C as illuminant source. Before each measurement, the apparatus was calibrated on the Hunterlab colour space system using a white ceramic tile (Minolta calibration plate, Y = 92.6, x = 0.3136, y = 0.3196). The Colour was described as Hue angle (H°, expressed as $\arctg\ b^*/a^*$) and Chroma (C, expressed as $(a^{*2} + b^{*2})^{1/2}$) indexes.

Weight loss: The weight loss was determined gravimetrically by weighing each PET tray at time zero and during the storage using a Technical balance (MP-3000 Chyo Balance corp., Japan). Changes in fruit weight were expressed as percentage of weight loss.

Purée preparation: For biophysical and chemical analyses, the strawberries (100g) were puréed using a handheld blender (Braun MR 4050 CA) for 30 s at high speed at each time of storage and kept frozen until the analyses were performed. The thawing was carried out overnight in a refrigerator at 5 ± 1 °C .

Dry matter content: Determinations were made on 5 g of fruit pulp by drying samples in an oven set at 105 °C. The samples were weighed after about 16 hours. The measurements were replicates three times. The results were expressed in g of dry matter over 100 g of samples.

Titrateable Acidity (TA): After the overnight thawing of samples at 4°C, TA was determined by titrating sample (2 g of homogenate + 40 mL of CO₂-free distilled water) with standardized 0.1 N NaOH to pH 8.2 (Phenolphthalein toning) by the use of a pH meter (Basic 20+, Crison Instruments SA, Barcelona, Spain). TA was expressed as citric acid equivalents (grams of citric acid per 100 grams of berries)

Total soluble solids (TTS): After the overnight thawing at 4°C, the berries pulp was put in the Automatic Refractometer model SMART-1 Atago®, (Atago CO.LTD, Tokyo, Japan). The results were expressed as BRIX°.

Respiration rate: The Apparent respiration rate (RR) of the strawberries was measured at 5 °C using the closed system method. The measurements were carried out in triplicate. The Berries and the jars were equilibrated for 1 h at 5°C. Samples of about 100 g were then placed in air in 0.5 L glass jars and tightly covered with metal caps equipped with silicone sampling ports. Headspace gas was periodically sampled (20-30 min) by means of a gas-tight syringe. Oxygen and carbon dioxide were detected and quantified through a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector and a steel column (2 m × 6 mm. CTR I Alltech, Milano), until the CO₂ level inside the jars reached 5%. The Respiration rate was calculated from the linear regression of O₂ and CO₂ concentrations measured during the experiment time and it was expressed in $ml*kg^{-1}*h^{-1}$.

$$RRO_2 = (\Delta[\%O_2]*V)/((\Delta t*100+M))$$

$$RRCO_2 = (\Delta[\%CO_2]*V)/((\Delta t*100+M))$$

Where:

V= Head space volume, ml

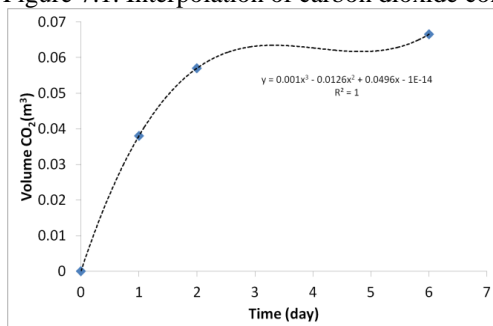
M= Mass of product, kg

t= Time, hour

Gases concentration: Headspace gas composition was periodically sampled through a gas-tight syringe. Oxygen and carbon dioxide were detected and quantified through a gas chromatography

(Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector (TCD) and a steel column (2 m x 6 mm CTR I Alltech, Milano). The GC oven was set isothermally at 50 °C. To better manage the evolution of carbon dioxide inside the master bag in the response surface methodology, the Volume of carbon dioxide was used as the response. The volume of carbon dioxide was estimated measurement the area below the best curve that interpolate the experimental points of carbon dioxide concentration multiplying the % with the head space volume (0.0095m³) as show in Figure 7.1. The results are expressed as m³ of carbon dioxide produced over the storage time.

Figure 7.1. Interpolation of carbon dioxide concentration evolution by best fit simple curve



Experimental plan

The Response Surface Methodology (RSM) was employed for optimizing the packaging conditions to extend the fruit shelf life. The relationship between responses (Visual acceptability and Score acceptability, Global acceptability, Maximum Force, Colour, Weight loss, Dry matter content, Titratable Acidity, °Brix and Gases concentration) and four independent variables: number of Oxygen Scavengers (N°), number of Carbon Dioxide Emitters (N°), ratio between Surface (S) and Unfilled Volume (UFV) expressed as cm²*cm⁻³ and, Time expressed as day (Table 7.2) were assessed. The results from the 26 runs design including all responses studied are shown in the Table 7.3 (1-2).

Table 7.2. Design of the experiment matrix for 4 Factors at 5 levels

Factors	Unit	Low Star	Low Cube	Center	High Cube	High Star
CO ₂ emitters *	N°	0	1	2	3	4
O ₂ scavengers **	N°	0	1	2	3	4
Ratio (Surface/UFV) ***	cm ² *cm ⁻³	0.352	0.362	0.367	0.372	0.377
Time	Day	2	6	10	14	18

*BioFresh® nominal capacity 500 cm³; ** CR-1FreshPax® Multisorb Technologies Inc., Buffalo, NY, USA; *** UFV: unfilled volume

Table 7.3. Experimental design matrix and responses 1/2

Run		CO ₂ EMITTERS	O ₂ SCAVENGERS	RATIO	TIME	Visual acceptability	Visual score	Global acceptability	Maximum force	Visual mouldy berries	Visual damaged berries
		N°	N°	cm ² *cm ⁻³	day	%	score	%	N	%	%
1	Low star	1	3	0.372	14	70	6.20	30.00	0.98	9.80	1.96
2	High star	3	3	0.372	14	60	6.10	45.00	0.99	5.56	3.70
3	Low star	2	4	0.367	10	50	5.83	60.00	0.99	0.00	1.92
4	High star	1	1	0.362	14	75	5.83	65.00	0.92	25.64	0.00
5	Low star	3	1	0.362	14	70	6.25	65.00	0.79	7.69	0.00
6	High star	1	1	0.372	14	75	6.38	70.00	0.84	8.20	1.64
7	Low star	1	1	0.372	6	60	6.08	55.00	1.06	1.89	0.00
8	High star	1	3	0.362	6	65	6.13	50.00	1.09	0.00	0.00
9	Fact	2	2	0.377	10	80	6.98	70.00	0.97	3.28	1.64
10	Fact	1	3	0.372	6	75	6.45	50.00	1.02	0.00	0.00
11	Fact	2	2	0.367	2	85	7.08	92.00	1.15	0.00	0.00
12	Fact	3	1	0.362	6	55	5.83	40.00	1.04	0.00	0.00
13	Fact	1	1	0.362	6	65	5.73	60.00	0.94	0.00	0.00
14	Fact	0	2	0.367	10	80	6.50	55.00	0.79	2.04	0.00
15	Fact	2	2	0.367	10	70	6.45	65.00	0.82	2.08	2.08
16	Fact	2	2	0.367	18	80	6.78	85.00	0.99	19.00	8.00
17	Fact	3	3	0.362	14	65	5.95	85.00	0.81	8.11	18.92
18	Fact	3	1	0.372	14	55	5.55	50.00	0.92	1.59	11.11
19	Fact	3	3	0.372	6	65	6.30	60.00	1.13	0.00	0.00
20	Fact	3	1	0.372	6	55	5.43	15.00	1.10	0.00	5.17
21	Fact	3	3	0.362	6	60	5.93	30.00	1.07	0.00	5.26
22	Fact	2	0	0.367	10	70	5.75	40.00	0.92	2.17	6.52
23	Fact	2	2	0.357	10	55	5.45	35.00	1.01	0.00	0.00
24	Center	1	3	0.362	14	55	5.80	45.00	1.10	6.82	0.00
25	Center	4	2	0.367	10	63	5.79	55.00	1.04	0.00	2.22
26	Center	2	2	0.367	10	85	6.78	70.00	0.97	7.69	0.00

Experimental design matrix and responses 2/2

Run		CO ₂ EMITTERS	O ₂ SCAVENGERS	RATIO	TIME	TA	TTS	Croma	Hue	Dry matter	Weight loss	O ₂	CO ₂	Quantity of CO ₂
		N°	N°	cm ² *cm ⁻³	day	g/100g	BRIX°	C*	H°	g/100g	%	%	%	m ³ *day
1	Low star	1	3	0.372	14	0.74	7.20	39.28	31.89	7.91	0.53	4.58	7.83	68.50
2	High star	3	3	0.372	14	0.73	6.40	43.86	34.60	6.98	0.86	5.95	6.21	106.50
3	Low star	2	4	0.367	10	0.76	7.85	38.32	30.84	8.49	0.85	7.56	5.95	65.30
4	High star	1	1	0.362	14	0.77	8.30	42.33	31.38	8.60	0.61	11.91	5.15	64.79
5	Low star	3	1	0.362	14	0.71	7.20	40.67	31.03	8.00	0.68	8.80	6.00	108.70
6	High star	1	1	0.372	14	0.68	7.65	42.13	31.86	8.32	0.48	4.95	6.20	76.40
7	Low star	1	1	0.372	6	0.82	7.35	41.25	31.82	8.33	0.29	13.05	5.77	31.10
8	High star	1	3	0.362	6	0.72	7.00	43.13	33.41	8.20	0.74	13.17	4.26	25.05
9	Fact	2	2	0.377	10	0.72	7.00	37.64	31.26	7.47	1.28	8.03	6.83	72.30
10	Fact	1	3	0.372	6	0.81	7.85	42.71	31.92	8.50	0.23	11.72	6.53	33.70
11	Fact	2	2	0.367	2	0.73	8.85	42.64	32.72	9.23	0.47	15.23	9.12	12.00
12	Fact	3	1	0.362	6	0.75	8.75	38.83	33.62	8.85	0.71	13.96	8.96	40.94
13	Fact	1	1	0.362	6	0.81	8.10	41.77	31.83	8.56	0.48	14.27	5.56	29.70
14	Fact	0	2	0.367	10	0.76	8.50	38.22	31.10	8.96	0.69	6.33	5.80	33.80
15	Fact	2	2	0.367	10	0.80	8.30	38.93	31.02	8.54	0.77	8.63	6.82	71.40
16	Fact	2	2	0.367	18	0.69	8.10	37.04	38.27	8.00	0.87	3.32	6.05	123.70
17	Fact	3	3	0.362	14	0.72	7.45	47.32	47.64	7.90	1.08	6.52	5.50	101.10
18	Fact	3	1	0.372	14	0.74	8.60	39.87	35.44	8.97	0.87	4.17	6.82	129.42
19	Fact	3	3	0.372	6	0.71	8.45	40.32	39.46	9.14	0.48	9.46	10.33	54.60
20	Fact	3	1	0.372	6	0.76	7.85	42.72	46.17	8.64	0.47	12.41	10.18	57.50
21	Fact	3	3	0.362	6	0.72	8.85	44.41	47.39	9.32	0.51	12.82	8.48	52.10
22	Fact	2	0	0.367	10	0.77	7.80	40.10	43.26	7.90	7.69	10.34	7.14	72.50
23	Fact	2	2	0.357	10	0.79	7.80	38.39	42.03	8.63	0.74	11.50	4.60	57.70
24	Center	1	3	0.362	14	0.83	6.45	43.87	49.05	7.64	0.72	6.07	4.88	60.20
25	Center	4	2	0.367	10	0.70	7.00	42.23	45.07	7.19	0.79	10.22	7.67	110.80
26	Center	2	2	0.367	10	0.75	7.05	40.36	45.77	7.46	0.55	10.11	5.48	67.30

7.4 RESULTS AND DISCUSSION

The initial characterization of strawberries is important to compare the evolution during storage among other experimental data.

The Respiration Rate (RR) is the most important index to define the “health” of fruit; in fact the Respiration Quotient (RQ) can be derived from it in order to verify the fruit metabolism. If the RQ is close to 1 it means that the fruit is using the sugar as feed energy.

The RR is useful to predict the carbon dioxide accumulation and the oxygen depletion during storage in a closed or semi-closed system. The production of carbon dioxide was $13 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ while the oxygen consumption was $15 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. The RQ was 0.88 that means the fruits were not in an optimal metabolic condition suggesting a mix of fat and carbohydrates as energetic resources.

Total soluble solids is one of the most important parameter to assess the fruits quality (Aday et al., 2011), and in traditional packaging condition this index decrease due to the hydrolysis of sucrose in order to maintain physiological activity and cell respiration (Aday et al., 2011; Li, Zhang, & Wang, 2008). The initial value was $7,80 \pm 0.01\%$ lower than some authors found (Peano et al., 2014)

7.4.1 Response Surface results

The response surface methodology (RSM) explores the relationships between several response of interest, y_1, y_2, y_i and a number of associated control (or input) variables denoted by x_1, x_2, x_k . The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. In general, a relationship is unknown but can be estimated and approximated by a low-degree polynomial model (e.g. linear or square; Teófilo and Ferreira, 2006). The application of RSM consists in an optimization technique following different steps: (1) select the independent variables of the main effects on the process studied taking into account the goal of the work; 2) choice the proper experimental design and carrying out the experiments according to the selected experimental matrix; 3) treat the data by using the mathematic–statistical techniques to obtain the best fitting through the polynomial function; 4) evaluate the model's fitting and (5) obtain the optimum results for each studied factors.

In this study, the relationship between response (fruits quality parameters and head space gases evolution) and four independent parameters (Oxygen scavengers, carbon dioxide emitters, ratio and time) were studied.

Using the Design Expert software, different models were fitted to the experimental responses.

7.4.1.1 Visual acceptability

The fit summary selected the quadratic model where the additional terms were significant and the model was not aliased.

Table 7.4 shows the ANOVA results. The statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted all data. The adjusted R^2 of 0.7333 and predicted R^2 of 0.5874 are satisfactory. Adequate precision of the model, which is a measure of signal to noise ratio (measure the model robustness), was 8.475 greater than 4 and indicated an adequate signal (the factors considered in the model were useful to predict the response).

The results of the ANOVA for Visual acceptability model showed that the four parameters and interaction A, BD, B², C² were significant model terms. The other terms were held in the models due to the respect of hierarchy of the terms, - hierarchy is ancestral linkage of effects flowing from main effects (regarded as parents) down through successive generations of higher-order interactions (children) - avoiding erroneous predictions when converted to actual terms (Peixoto, 1990). If C² is significant it is not possible to delete the factor C, because removing the term it would reduce the precision and accuracy of the model.

This model can be reduced somewhat by eliminating the insignificant interaction terms AD, CD and quadratic terms D² (p>0.1). Some statisticians caution against such elimination of specific terms, advocating instead that modelers maintain the entire quadratic polynomial as standard practice (Myers and Montgomery, 2002). However, in this case by taking out terms the predicted, R² increases from 0.27 to 0.58.

Table 7.4. ANOVA for response surface reduced quadratic model.

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remarks</i>
Model	2051.96	11	186.54	6.75	0.0013	<i>significant</i>
A-CO2 emitters	330.04	1	330.04	11.94	0.0048	
B-O2 scavengers	1.74	1	1.74	0.063	0.8062	
C-Ratio	1.58	1	1.58	0.057	0.8149	
D-Time	9.38	1	9.38	0.34	0.5711	
AB	39.06	1	39.06	1.41	0.2575	
AC	76.56	1	76.56	2.77	0.1219	
BC	126.56	1	126.56	4.58	0.0536	
BD	189.06	1	189.06	6.84	0.0226	
A²	98.60	1	98.60	3.57	0.0833	
B²	744.44	1	744.44	26.93	0.0002	
C²	472.06	1	472.06	17.08	0.0014	
Residual	331.66	12	27.64			
Lack of Fit	219.16	11	19.92	0.18	0.9633	<i>not significant</i>
Pure Error	112.50	1	112.50			

The final model in terms of coded factors is shown in Equation 7.1. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Greater the number greater the influence.

In this case the number of carbon dioxide and the quadratic factors of the oxygen scavenger and ratio perform the most important factors for this quality index. The coefficients for each factor represent in the equation 7.1 define the effect on the model. Positive value of coefficients establish that increasing the value of the factor (e.g. number of oxygen scavenger) will be generated an increase on the response (e.g. visual acceptability), on the contrary negative coefficient generate a decrease of the response value. Increasing the number of oxygen scavengers and the Time of storage and, the amount of product (Ratio) the Visual acceptability value increases while increasing the numbers of carbon dioxide emitters the berries show a decrease in acceptance by the consumer.

Equation 7.1.

$$\text{Visual Acceptability} = +79.9 - 3.7 * A + 0.3 * B + 0.3 * C + 0.6 * D + 1.6 * AB - 2.2 * AC + 2.8 * BC - 3.4 * BD - 2.1 * A^2 - 7.7 * B^2 - 6.1 * C^2$$

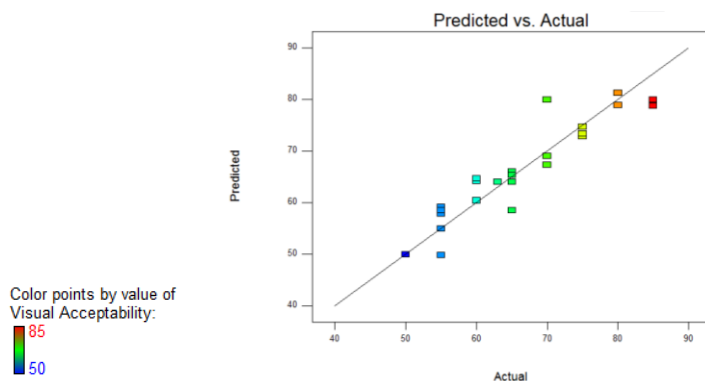
The final model in terms of actual factors is shown in Equation 7.2. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Equation 7.2.

$$\text{Visual Acceptability} = -32805.2 + 162.2 * \text{CO2emitters} - 169.9 * \text{O2scavengers} + 1.8E+5 * \text{Ratio} + 1.9 * \text{Time} + 1.6 * \text{CO2 emitters} * \text{O2 scavengers} - 437.5 * \text{CO2 emitters} * \text{Ratio} + 562.5 * \text{O2 scavengers} * \text{Ratio} - 0.9 * \text{O2 scavengers} * \text{Time} - 2.1 * \text{CO2 emitters}^2 - 7.7 * \text{O2 scavengers}^2 - 2.4E+5 * \text{Ratio}^2$$

Figure 7.2 shows the relationship between the actual and predicted values of the visual acceptability. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.2. Scatter diagram for Visual Acceptability



One of the RSM advantages over the one factor at a time experimental procedure is its ability to specify the interaction effect between any two factors. Figure 7.3 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different Ratio at 10 days of storage. It is clear from the figures that a master bag with an average amount of the product (750g) determine an higher visual acceptability from consumers. In this conditions (Figure 3 b), the maximum acceptability (more than 80%) occurs at a combination of about 2 oxygen scavengers and from 1 to 2 carbon dioxide emitters.

The Figure 7.4 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and the number of carbon dioxide emitters considering the average ratio and different storage times a) 10 days b) 14 days.

The same combination of active devices (using average ratio) proposed above maintain the visual acceptability more than 80% of oxygen scavenger also at 14 days of storage.

Figure 7.3. 2D contour plot of Visual Acceptability at 10 days of storage with different Ratio a) 0.362 b) 0.367 and c) 0.372.

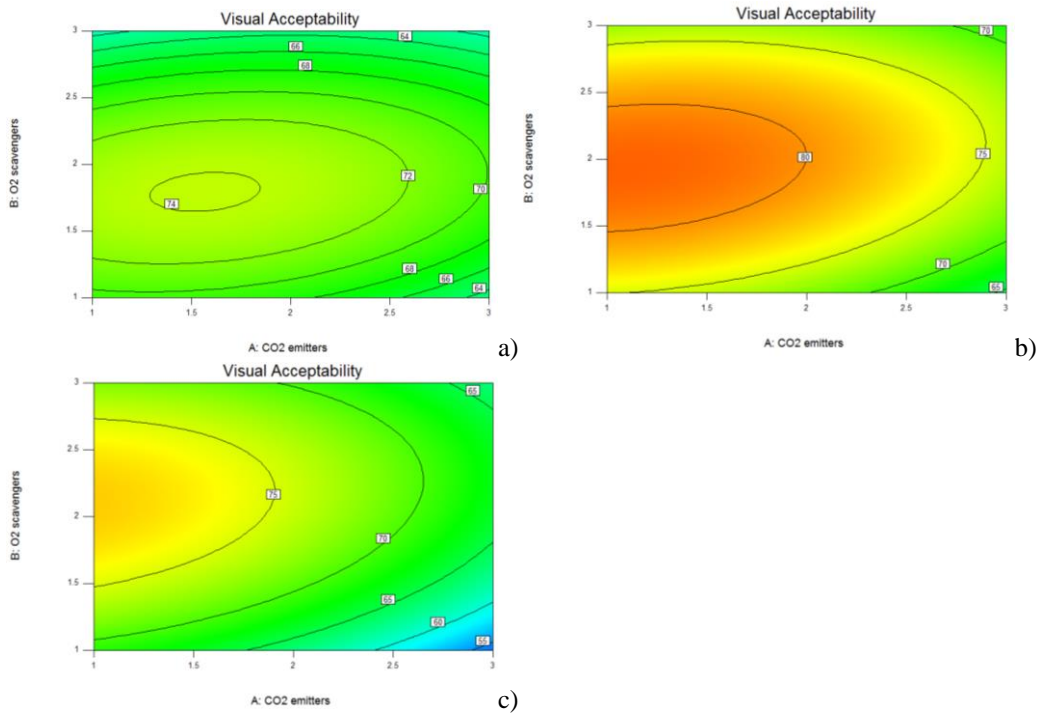
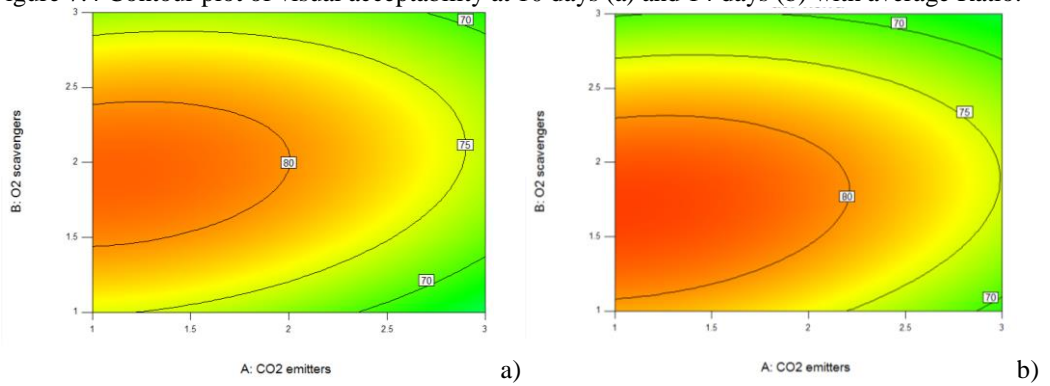


Figure 7.4 Contour plot of visual acceptability at 10 days (a) and 14 days (b) with average Ratio.



7.4.1.2 Score acceptability

As a result of analyzing the responses using the specific software, the fit summary selected the quadratic model where the additional terms are significant and the model is not aliased.

Table 7.5 presents the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted all data. The adjusted R² of 0.7559 and predicted R² of 0.5381 are satisfactory. Adequate precision of the model, which is a measure of signal to noise ratio, was 8.475 greater than 4 and indicated an adequate signal.

The results of the ANOVA for score acceptability model shows that the four parameters and interaction A, AC, A², B², C² were significant model terms.

This model can be reduced somewhat by eliminating some insignificant interaction terms CD, AB, AD and D² (p>0.1). However, in this case by taking out terms the predicted R² increases from 0.26 to 0.53.

Table 7.5. ANOVA for response surface reduced quadratic model.

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remark</i>
Model	3.71	10	0.37	8.43	0.0002	significant
A-CO2 emitters	0.30	1	0.30	6.78	0.0208	
B-O2 scavengers	0.16	1	0.16	3.57	0.0795	
C-Ratio	0.051	1	0.051	1.15	0.3009	
D-Time	6.803E-003	1	6.803E-003	0.15	0.7003	
AC	0.30	1	0.30	6.83	0.0204	
BC	0.13	1	0.13	3.01	0.1048	
BD	0.18	1	0.18	4.13	0.0616	
A²	0.57	1	0.57	12.98	0.0029	
B²	1.38	1	1.38	31.28	< 0.0001	
C²	1.34	1	1.34	30.48	< 0.0001	
Residual	0.62	14	0.044			
Lack of Fit	0.56	13	0.043	0.82	0.7101	not significant
Pure Error	0.053	1	0.053			

The final model in terms of coded factors is shown in Equation 7.3.

Equation 7.3.

$$\text{Visual Score} = +6.75 - 0.11 * A + 0.081 * B + 0.054 * C - 0.02 * D - 0.14 * AC + 0.09 * BC - 0.11 * BD - 0.16 * A^2 - 0.25 * B^2 - 0.32 * C^2$$

The final model in terms of actual factors is shown in Equation 7.4.

Equation 7.4

$$\text{Visual Score} = -1726.0 + 10.6 * \text{CO2 emitters} - 5.3 * \text{O2 scavengers} + 9402.4 * \text{Ratio} + 0.05 * \text{Time} - 27.4 * \text{CO2 emitters} * \text{Ratio} + 18.2 * \text{O2 scavengers} * \text{Ratio} - 0.03 * \text{O2 scavengers} * \text{Time} - 0.2 * \text{CO2 emitters}^2 - 0.2 * \text{O2 scavengers}^2 - 12769.9 * \text{Ratio}^2$$

The coefficients for each factor represent in the equation 7.3 define the effect on the model.

The negative quadratic factors of the model show an important effect in the prediction of the score acceptability identifying a maximum area on the response surface.

Figure 7.5 shows the relationship between the actual and predicted values of the score acceptability. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.5. Scatter diagram for Score Acceptability

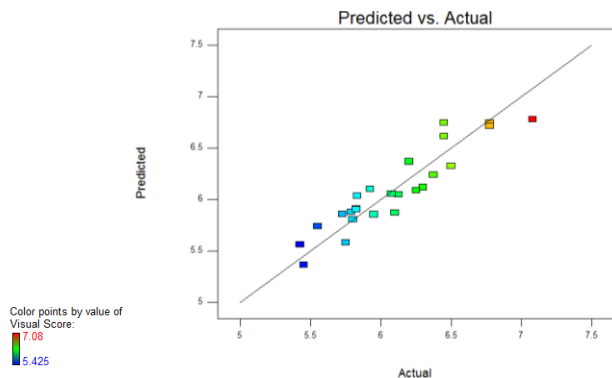
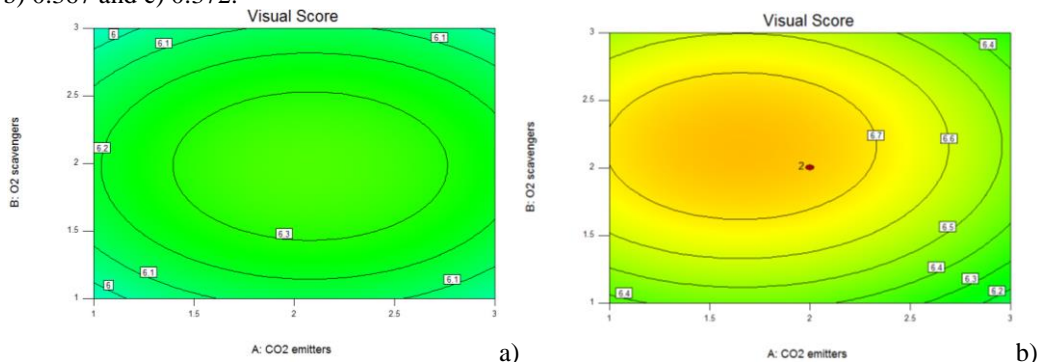


Figure 7.6 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different Ratio at 10 days. In this condition (Figure 7.6b), the maximum acceptability (more than 6.7) occurs at a combination of about 2-2.5 oxygen scavengers and from 1.5 to 2 carbon dioxide emitters.

The Figure 7.7 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters considering an average ratio value but at different times a) 10 days b) 14 days. The same combination of active devices (using average ratio) proposed above maintain the visual acceptability more than 80% of oxygen scavenger also at 14 days of storage.

Figure 5. 2D contour plot of Score Acceptability at 10 days of storage with different Ratio a) 0.362 b) 0.367 and c) 0.372.



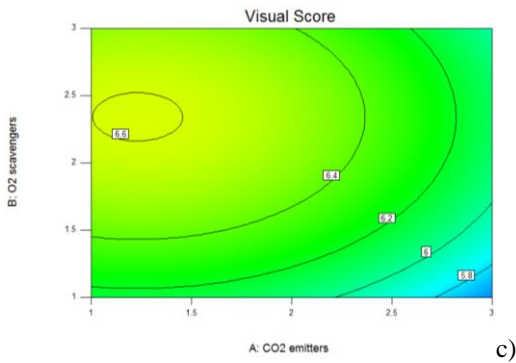
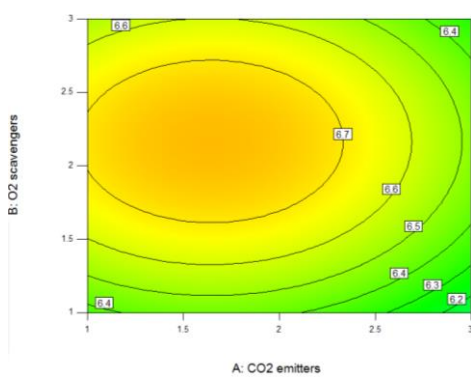
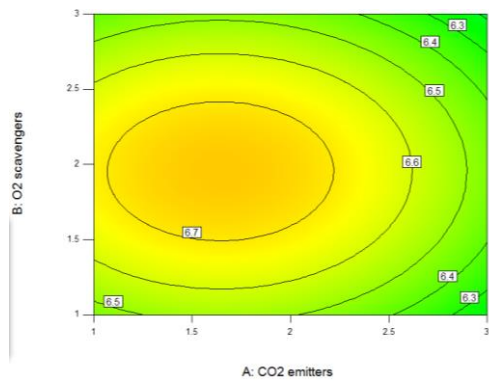


Figure 5.1. Contour plot of score acceptability at 10 days (a) and 14 days (b) with average Ratio.



a)



b)

7.4.1.3 Global Acceptability

As a result of analyzing the responses using the specific software, the fit summary selected the quadratic model where the additional terms are significant and the model is not aliased.

Table 5 shows the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted to all data. The adjusted R^2 of 0.8804 and predicted R^2 of 0.7037 are closed to each other which are satisfactory. Adequate precision of the model, which is a measure of signal to noise ratio, was 15.407 greater than 4 and indicated an adequate signal.

The results of the ANOVA for score acceptability model shows that the four parameters and interaction B, C, AB, AC, BD, B^2 , C^2 , D^2 were significant model terms. The other terms were held in the models due to the respect of hierarchy of the terms. However, in this case by taking out terms the predicted R^2 increases from 0.63 to 0.70.

Table 7.5. ANOVA for response surface reduced quadratic model.

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remark</i>
Model	6942.92	13	534.07	14.02	0.0001	<i>significant</i>
A-CO2 emitters	26.07	1	26.07	0.68	0.4274	
B-O2 scavengers	221.77	1	221.77	5.82	0.0365	
C-Ratio	925.29	1	925.29	24.29	0.0006	
D-Time	15.19	1	15.19	0.40	0.5419	
AB	1912.63	1	1912.63	50.21	< 0.0001	
AC	509.95	1	509.95	13.39	0.0044	
AD	106.50	1	106.50	2.80	0.1254	
BC	117.32	1	117.32	3.08	0.1098	
BD	878.09	1	878.09	23.05	0.0007	
A^2	144.91	1	144.91	3.80	0.0797	
B^2	295.79	1	295.79	7.77	0.0192	
C^2	1598.52	1	1598.52	41.97	< 0.0001	
D^2	508.08	1	508.08	13.34	0.0044	
Residual	380.91	10	38.09			<i>not significant</i>
Lack of Fit	368.41	9	40.93	3.27	0.4060	
Pure Error	12.50	1	12.50			

The final model in terms of coded factors is shown in Equation 7.5.

In this case the interaction between the number of oxygen scavengers and carbond dioxide emitters and the quadratic effect of the Ratio determines the main effects on the changes of global quality of strawberries during storage. The numbers of carbon dioxide emitters, the number of oxygen scavengers and, the time have a positive effect on global acceptability while the Ratio have a negative impact on the quality index.

Equation 7.5.

$$\text{Global acceptability} = +67.5 + 1.1 * A + 3.2 * B - 7.7 * C + 0.8 * D + 11.6 * AB - 6.0 * AC + 2.7 * AD - 2.9 * BC - 7.9 * BD - 2.9 * A^2 - 4.2 * B^2 - 11.5 * C^2 + 5.5 * D^2$$

Equation 7.6.

$$\text{Global acceptability} = -62839.3 + 423.4 * \text{CO2 emitters} + 227.7 * \text{O2 scavengers} + 3.4E+5 * \text{Ratio} - 4.0 * \text{Time} + 11.6 * \text{CO2 emitters} * \text{O2 scavengers} - 1201.1 * \text{CO2 emitters} * \text{Ratio} + 0.7 * \text{CO2 emitters} * \text{Time} - 576.1 * \text{O2 scavengers} * \text{Ratio} - 2.0 * \text{O2 scavengers} * \text{Time} - 2.9 * \text{CO2 emitters}^2 - 4.1 * \text{O2 scavengers}^2 - 4.6E+5 * \text{Ratio}^2 + 0.3 * \text{Time}^2$$

Figure 7.8. Shows the relationship between the actual and predicted values of the Global acceptability. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.8. Scatter diagram for Global acceptability

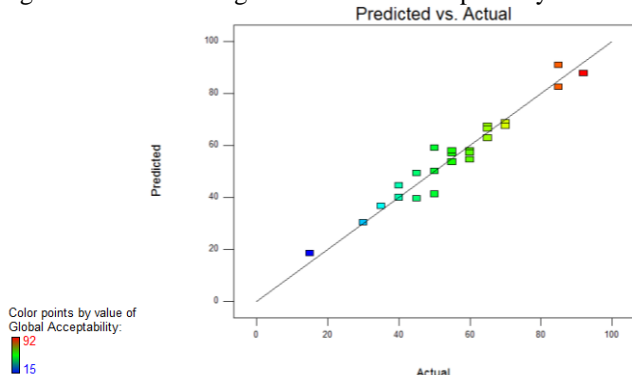
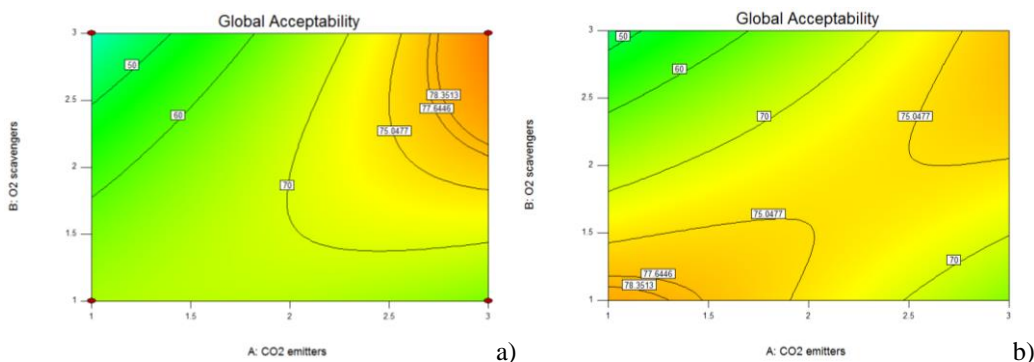


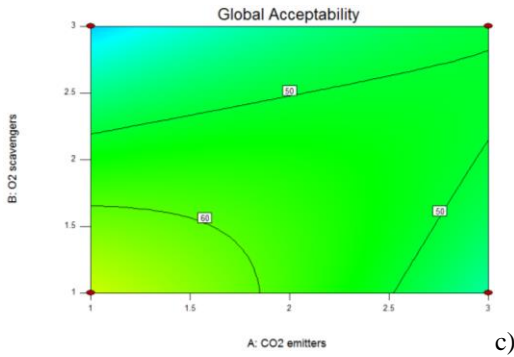
Figure 7.9 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different Ratio at 14 days. In the conditions with low Ratio value (Figure 7.9a), the maximum level of global acceptability occurs at a combination of about 3 oxygen scavengers and 3 carbon dioxide emitters. Whereas using the average value of Ratio the Global acceptability reached the similar value in two different combinations, using about 1 oxygen scavenger and 1 carbon dioxide emitter or using 3 oxygen scavengers and 3 carbon dioxide emitters. Taking into account the cost of the active devices and the results obtained for the other visual indicators, it is clear that the first choice of this solution should be used.

On the contrary the higher quantity of products inside the master bag can generate an incorrect gases evolution in the head space, in terms of low oxygen and high carbon dioxide concentration, and determine a lower Global acceptability at the end of the storage.

The difference in the combination of active devices (oxygen scavenger and carbon dioxide emitter) for the global acceptability respect to the visual acceptability can be explained by the different taste (bitterness or fermented) evaluated by the consumer.

Figure 7.9. 2D contour plot of Global Acceptability at 14 days of storage with different Ratio a) 0.362 b) 0.367 and c) 0.372.

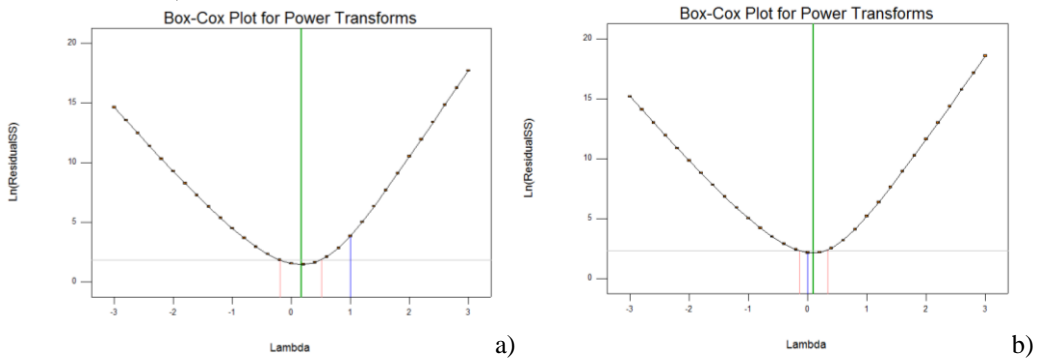




7.4.1.4 Visual mouldy berries

The primary statistical tool for identifying the need for transformations and for pinpointing which one works best, is the Box-Cox plot, which Anderson and Whitcomb (2005) detail in their Chapter 5 appendix. The Box-Cox plot is a tool to help in determining the most appropriate power transformation to apply to the response data. The plot shows the minimum lambda values, as well as lambdas at the 95% confidence range. The plot also shows the current power transformation so you can see where that fits. In this case, as showed in Figure 7.9a, the fit went over the limit and the software suggested a logarithmic transformation (Log10). In figure 7.9b the results of this transformation was shown.

Figure 7.9: Percentage of visual mouldy berries without transformation a) and with logarithmic transformation b)



As a result of analyzing the responses using the specific software, the fit summary selected the linear model where the additional terms are significant and the model is not aliased.

Table 7.6 shows the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted to all data. The adjusted R^2 of 0.6954 and predicted R^2 of 0.6181 are satisfactory. Adequate precision of the model, which is the measure of signal to noise ratio, was 13.213 greater than 4 and indicated an adequate signal.

The results of the ANOVA for Percentage of visual mouldy model shows that only the factor D was a significant model term. The other terms were held in the models because they improved the prediction of the model on the quality index.

Table 7.6. ANOVA for response surface linear model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remark
Model	27.46	4	6.86	14.70	< 0.0001	significant
A-CO₂ emitters	1.96	1	1.96	4.19	0.0539	
B-O₂ scavengers	1.25	1	1.25	2.68	0.1174	
C-Ratio	1.30	1	1.30	2.79	0.1104	
D-Time	23.24	1	23.24	49.77	< 0.0001	
Residual	9.34	20	0.47			
Lack of Fit	9.18	19	0.48	3.03	0.4274	not significant
Pure Error	0.16	1	0.16			

The final model, in terms of coded factors, is shown in Equation 7.7.

Equation 7.7.

$$\text{Log}_{10}(\text{Percentage of visual mouldy berries} + 0.02) = -0.3 - 0.3 * A - 0.2 * B + 0.2 * C + 1.0 * D$$

Equation 7.8.

$$\text{Log}_{10}(\text{Percentage of visual mouldy berries} + 0.02) = -19.3 - 0.3 * \text{CO}_2 \text{ emitters} - 0.2 * \text{O}_2 \text{ scavengers} + 47.8 * \text{Ratio} + 0.2 * \text{Time}$$

Analysing the equation 7.7, an increase on the number of carbon dioxide emitters and the number of oxygen scavengers produced a adverse gases concentration condition in the head space of the master bag reducing the mould growth, while during storage and with higher amount of berries inside the master bag the mould have found the favourable condition to grow. in terms of the gases concentration reaching the advantageous situation to growth the mould on the berries surface.

Figure 7.10 shows the relationship between the actual and predicted values of the visual mouldy berries. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.10. Scatter diagram for Visual mouldy berries

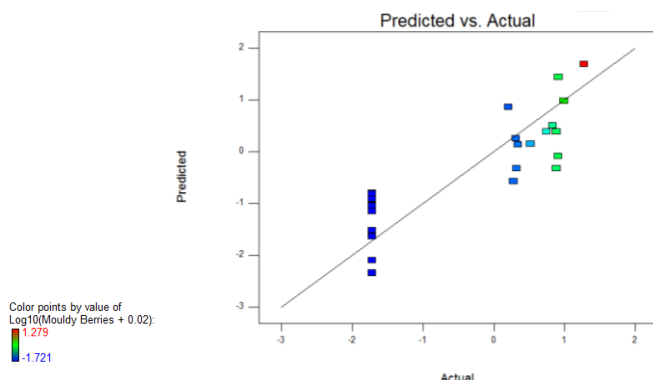
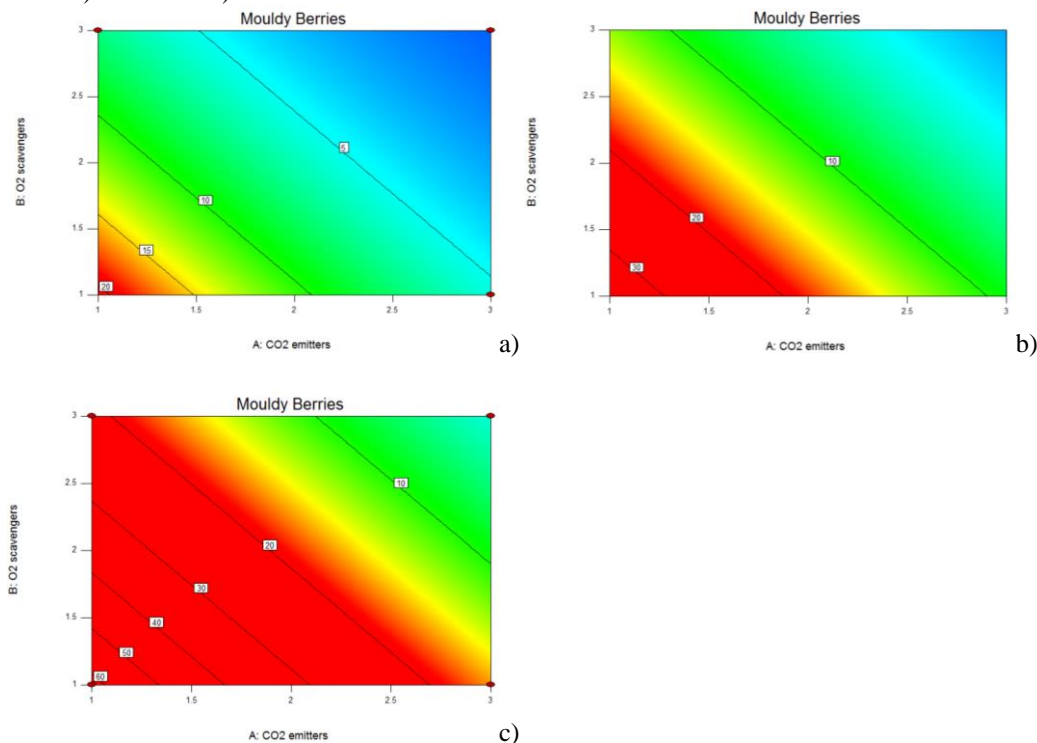


Figure 7.11 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different Ratio at 14 days. It is clear that using a low value of Ratio, the quality of berries (in terms of mouldy growth) was maintain longer. In particular, using a low Ratio value (Figure 7.11), the combination with more than 2 oxygen scavengers and 2 carbon dioxide emitters allows a low value of percentage of mouldy berries, keeping the value under the limit (5% Hertog et al., 1999). Increasing the amount of product the berries generate a optimal condition for mould growth in terms of the high humidity inside the master bag and the inappropriate gases concentration.

Figure 7.11. 2D contour plot of Visual mouldy berries at 14 days of storage with different Ratio a) 0.362 b) 0.367 and c) 0.372.



7.4.1.5 Concentration of Oxygen inside the master bag

As a result of analyzing the responses using the specific software, the fit summary selected the linear model where the additional terms are significant and the model is not aliased.

Table 7.7 shows the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted to all data. The adjusted R^2 of 0.9132 and predicted R^2 of 0.9022 are closed to each other which are satisfactory. Adequate precision of the model, which is the measure of the signal to noise ratio, was 30.745 greater than 4 and indicated an adequate signal.

The results of the ANOVA for score acceptability model shows that the four parameters and interaction B, C, D were significant model terms. As expected the number of carbon dioxide emitters had insignificant effect on this parameters.

Table 7.7. ANOVA for response surface reduced linear model

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remark</i>
Model	270.61	3	90.20	85.14	< 0.0001	<i>significant</i>
B-O2 scavengers	8.45	1	8.45	7.98	0.0102	
C-Ratio	22.79	1	22.79	21.51	0.0001	
D-Time	228.94	1	228.94	216.10	< 0.0001	
Residual	22.25	21	1.06			<i>not significant</i>
Lack of Fit	21.15	20	1.06	0.97	0.6790	
Pure Error	1.10	1	1.10			

The final model in terms of coded factors is shown in Equation 7.9. The factor time and the Ratio define an important effect on the model. This result is correlated with the consumption of the oxygen by the fruits, increasing the amount of berries inside the master bag the oxygen value decrease quickly (High Ratio) and the metabolism of the fruit continues to decrease the concentration of oxygen in the package during storage

Equation 7.9.

$$\text{Oxygen concentration} = +9.3 - 0.6 * B - 1.0 * C - 3.2 * D$$

The final model in terms of actual factors is shown in Equation 7.10.

Equation 7.10

$$\text{Oxygen concentration} = +91.7 - 0.6 * \text{O2 scavengers} - 199.7 * \text{Ratio} - 0.8 * \text{Time}$$

The equation 7.09 shows that the all factors have a negative influence on oxygen concentration, in fact during storage and with higher amount of product the oxygen decrease the concentration due to the fruit respiration. As expected The increasing of the number of scavenger decrease the amount of the oxygen in head space.

Figure 7.12 shows the relationship between the actual and predicted values of the oxygen concentration. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.12. Scatter diagram for oxygen concentration

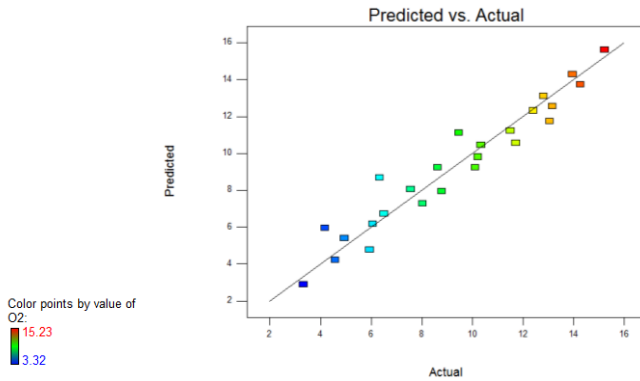
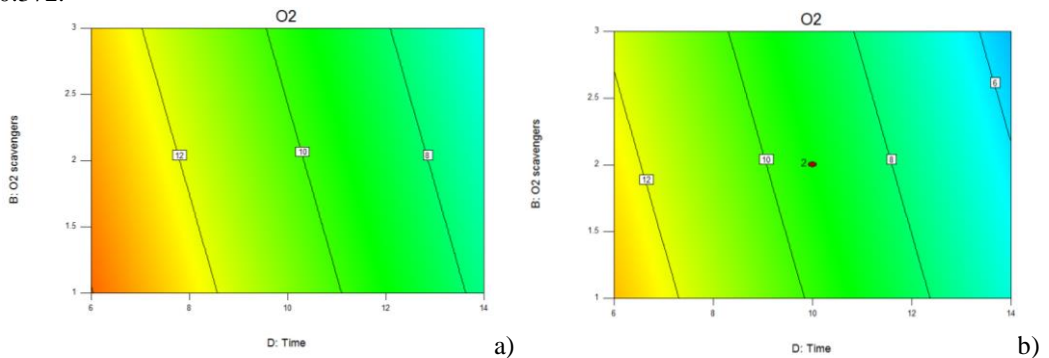
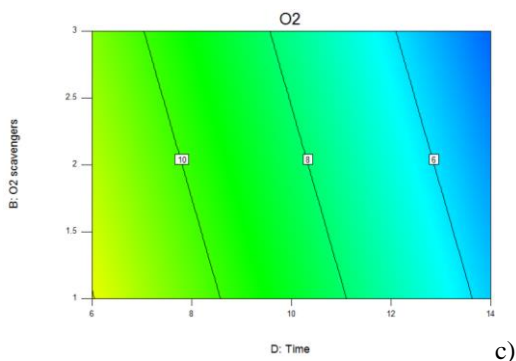


Figure 7.13 shows the 2-D contour graphs, highlighting the interaction number of oxygen scavenger and Time. It is clear from figures that during storage the oxygen concentration decrease and the presence inside the master bag of fruit accelerates this phenomena due to the respiration of berries. In fact at higher Ratio value the oxygen value went under 2% at the end of the storage. For some authors, this value has been considered as the limit at which the fruit develops an injury (Beaudry 2000 and Kader et al., 1989).

The oxygen scavenger had a rule in the depletion of oxygen as function of the number of device due to the scavenging capacity that each device can capture. This conclusion is well demonstrated in the figures by the slightly rate of the iso-response towards the number of oxygen scavenger. It is clear that higher is the number of scavenger, lower is the oxygen value.

Figure 7.13. 2D contour plot of oxygen concentration with different Ratio a) 0.362 b) 0.367 and c) 0.372.





7.4.1.6 CO₂ concentration

As a result of analyzing the responses using the specific software, the fit summary selected the 2 factor interaction model where the additional terms are significant and the model is not aliased.

Table 8 shows the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted to all data. The adjusted R² of 0.8053 and predicted R² of 0.7448 are closed to each other which are satisfactory. Adequate precision of the model, which is a measure of the signal to noise ratio, was 14.650 greater than 4 and indicated an adequate signal.

The results of the ANOVA for CO₂ concentration model shows that the four parameters and interaction A, C, D and AD were significant model terms. The other terms were held in the models due to the respect of hierarchy of the terms.

Table 7.8. ANOVA for response surface reduced 2 factor interaction model.

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remark</i>
Model	56.12	6	9.35	18.23	< 0.0001	<i>significant</i>
A-CO2 emitters	16.72	1	16.72	32.59	< 0.0001	
B-O2 scavengers	0.38	1	0.38	0.73	0.4032	
C-Ratio	10.06	1	10.06	19.62	0.0003	
D-Time	12.95	1	12.95	25.25	< 0.0001	
AD	14.76	1	14.76	28.79	< 0.0001	
BC	1.25	1	1.25	2.43	0.1352	
Residual	9.75	19	0.51			
Lack of Fit	8.85	18	0.49	0.55	0.8067	<i>not significant</i>
Pure Error	0.90	1	0.90			

The final model in terms of coded factors is shown in Equation 7.11.

In this case the interaction between Time and number of carbon dioxide emitters showed a highest influence on the CO₂ concentration. This can be explain due to the kinetics of CO₂ production from the emitter device, thus, increasing the time the gas production increases too. This equation shows that also the Ratio can have a effect on the model, due to the respiration of the fruit that produce carbon dioxide inside the master bag.

Equation 7.11.

$$\% \text{ CO}_2 = +6.7 + 0.8 * A - 0.1 * B + 0.6 * C - 0.7 * D - 1.0 * AD + 0.3 * BC$$

The final model in terms of actual factors is shown in Equation 7.2.

Equation 7.12.

$$\text{CO}_2 = -4.2 + 3.2 * \text{CO}_2 \text{ emitters} - 20.6 * \text{O}_2 \text{ scavengers} + 17.7 * \text{Ratio} - 0.3 * \text{Time} - 0.2 * \text{CO}_2 \text{ emitters} * \text{Time} + 55.9 * \text{O}_2 \text{ scavengers} * \text{Ratio}$$

Figure 7.13 shows the relationship between the actual and predicted values of the CO₂ concentration. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.13. Scatter diagram for CO₂ concentration

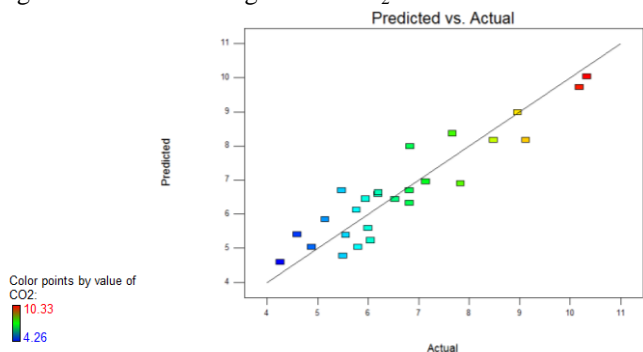


Figure 7.14 shows the 2-D contour graphs, highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different Ratio at 10 days. It is clear from figures that the increasing in the amount of the fruit (Ratio) inside the master bag, the carbon dioxide concentration increase due to the respiration of the berries. The emitter device has a high effect on the carbon dioxide concentration, in fact in Figures 7.14, the iso-responses have an orthogonal trend respect on the increase of the emitter device numbers. A slightly effect by the oxygen scavenger device could be observed especially in Figure 13c, where the amount of the fruits inside the master bag was lower and the effect of carbon dioxide scavenging by the oxygen scavenger was highlighted due to the presence of silica gel inside the formulation that could absorb also the CO₂.

Figure 7.15 shows the 2D contour graphs highlighting the interaction effect between the number of oxygen scavengers and number of carbon dioxide emitters at different times, considering an average Ratio value, to demonstrate the effect of time on the evolution in the CO₂ concentration. In fact, with respect of the Figure 7.15b, this figure shows lower value indicating that during storage the CO₂ decrease due to the permeation of gas through the plastic film and the depletion of the carbon dioxide emitter.

The carbon dioxide concentration has an important role to extend or maintain the fruit quality in packaging system. The optimal concentration of carbon dioxide is established between 15-20% (Kader 1980 and 1992). This range can change as function of the storage temperature, cultivar, fruits healthiness and the concentration of carbon dioxide. In other works (Kader and Salveit, 2002

book; Kader 1997) the concentration to inhibit the mould growth start from 10% : this concentration may avoid the fermentation flavour production that may be perceived by the consumer as off-flavour. To maintain the minimum concentration to inhibit the mould growth also at 10 day of storage it is necessary increase the amount of the berries inside the master bag with highest number of CO₂ emitter taking into consideration the effect of gases production by emitters in the initial part of the storage. In fact the range of interest of DOE modelled the evolution of carbon dioxide from 6 to 14 days, this interval didn't show the peak of production by the carbon dioxide emitters at beginning of the experiment. The exceed of the limit (>25%) also for short time can contribute to generate off-flavour and damaged on the berries surface. For these reason the modelling of only the CO₂ concentration didn't allow to evaluate the evolution at initial period of storage. In the following paragraph the Volume of CO₂ was used to model more efficiency the evolution of this gas inside the master bag.

Figure 7.14. 2D contour plot of carbon dioxide concentration at 10 days of storage with different Ratio a) 0.362 b) 0.367 and c) 0.372.

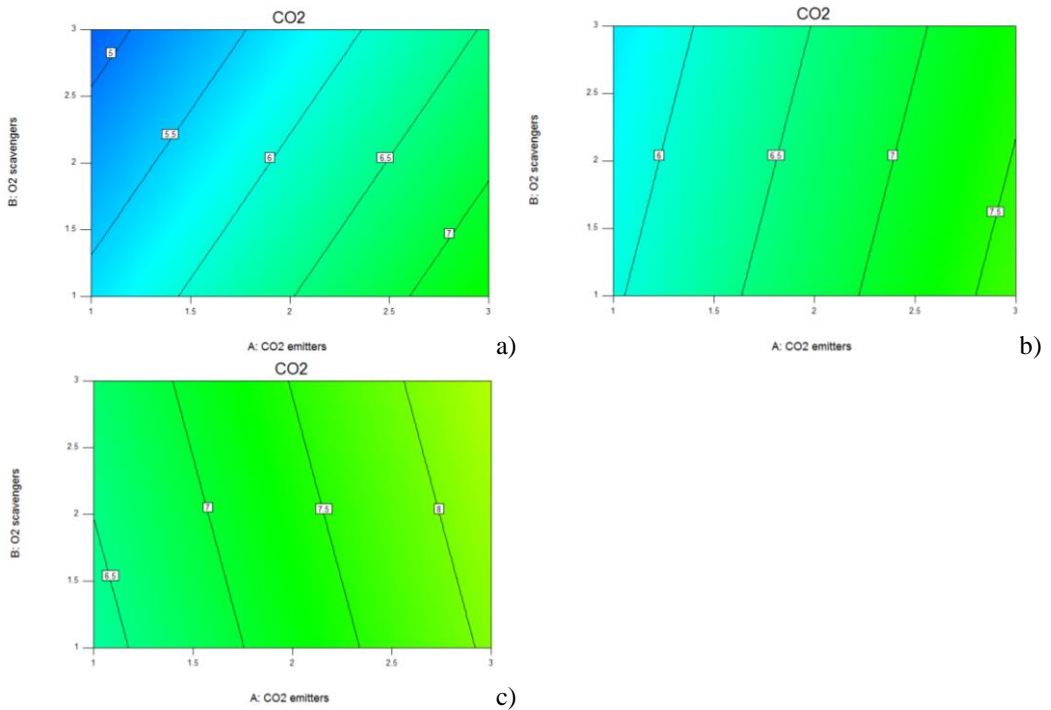
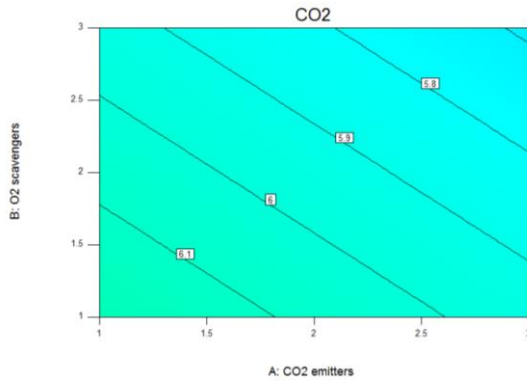


Figure 7.15. 2D contour plot of carbon dioxide concentration at 14 days of storage with 0.365 as Ratio



7.4.1.7 Volume of Carbon Dioxide

As a result of analyzing the responses using the specific software, the fit summary selected the 2-factor interactions model where the additional terms are significant and the model is not aliased. Table 9 shows the ANOVA results and as it can be seen the statistical analysis of variance revealed an overall model p-value (probability of error value) less than 0.05 which is significant. On the other hand, lack of fit testing produced a p-value greater than 0.01 that indicates the model well fitted to all data. The adjusted R^2 of 0.9746 and predicted R^2 of 0.9626 are closed to each other which are satisfactory. Adequate precision of the model, which is the measure of the signal to noise ratio, was 42.139 greater than 4 and indicated an adequate signal.

The results of the ANOVA for Volume of CO_2 model shows that all parameters were significant

Table 7.9. ANOVA for response surface reduced 2-factor interactions model.

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F Value</i>	<i>p-value Prob > F</i>	<i>Remark</i>
Model	2.18	6	0.36	161.01	< 0.0001	significant
A-CO2 emitters	0.65	1	0.65	287.78	< 0.0001	
B-O2 scavengers	9.858E-003	1	9.858E-3	4.37	0.0502	
C-Ratio	0.041	1	0.041	18.15	0.0004	
D-Time	1.42	1	1.42	629.32	< 0.0001	
AD	0.046	1	0.046	20.37	0.0002	
BD	0.014	1	0.014	6.06	0.0236	
Residual	0.043	19	2.255E-3			
Lack of Fit	0.042	18	2.338E-3	3.08	0.4240	not significant
Pure Error	7.586E-004	1	7.586E-4			

The final model in terms of coded factors is shown in Equation 7.13. In this case, as expected, the time and the number of carbon dioxide emitters perform the main factors in the increase of carbon dioxide inside the master bag, due to the initial production of the device that gives a great contribute in the carbon dioxide concentration in the head space.

All factor have a positive influence of Volume of CO_2 due to the effect of emitters device and respiration of the fruits inside the master bag. Only the interaction between oxygen scavenger and

time shows a negative effect on the volume of CO₂, probably due to the absorption of the carbon dioxide gas, during storage, from silica gel contained inside the oxygen scavenger device.

Equation 7.13.

$$\text{Volume of CO}_2 = +0.6 + 0.2 * A - 0.02 * B + 0.04 * C + 0.2 * D + 0.05 * AD - 0.03 * BD$$

The final model in terms of actual factors is shown in Equation 7.14

Equation 7.14

$$\text{Volume of CO}_2 = -3.2 + 0.03 * \text{CO}_2 \text{ emitters} + 0.05 * \text{O}_2 \text{ scavengers} + 8.2 * \text{Ratio} + 0.05 * \text{Time} + 0.01 * \text{CO}_2 \text{ emitters} * \text{Time} - 7.3\text{E-}3 * \text{O}_2 \text{ scavengers} * \text{Time}$$

Figure 7.16 shows the relationship between the actual and predicted values of the Volume of CO₂. This figure indicates that the developed model is adequate, since the residuals in the prediction of each response are small, with the residuals tending to be close to the diagonal line.

Figure 7.16. Scatter diagram for Volume of CO₂

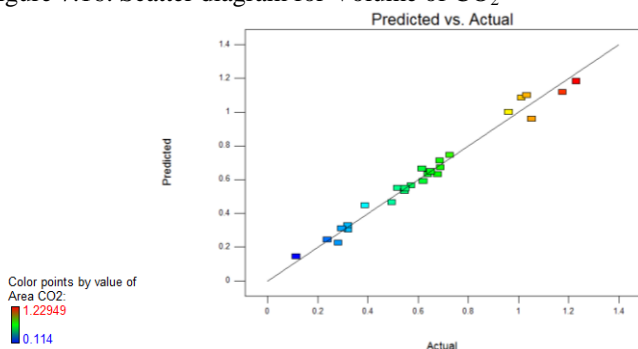
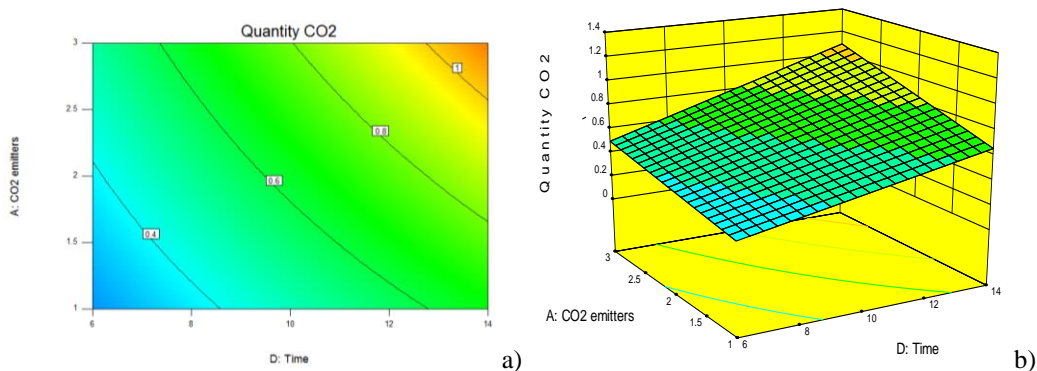


Figure 7.17 shows the 2-D contour and 3D surface graphs, highlighting the interaction number of carbon dioxide emitter and Time. It is clear from figures that during storage the carbon dioxide quantity increases due to the respiration of fruit and the carbon dioxide released by the emitters inserted into the master bag and the synergistic effect of combination between the number of carbon dioxide emitter and time.

Figure 7.17. 2D a) contour plot and 3D surface of Volume of carbon dioxide



There were other quality parameters studied in this work such as, Colour indices, Total Solid Soluble ($^{\circ}$ brix), Total Solid (g/100g) and Titratable Acidity (g/100g) but no one resulted significant on the evolution of strawberries quality.

Moreover, the percentage of fruits weight loss had an effect on the quality of fruits but this effect was comparable in all the tested conditions because the master bags were made by the same materials that offered a right water vapour barrier and maintained the correct value of relative humidity into the packaging. For this reasons the weight loss was not used in the optimization step.

7.4.2 Optimization of the selected parameters

Eleven criteria (CO_2 emitters; O_2 scavengers; Ratio; Time; Visual Acceptability; Visual Score; Global Acceptability; Visual Mouldy Berries; O_2 concentration; CO_2 concentration; Volume of CO_2) were introduced in the numerical optimization. The first four criteria were correlated the factors used in the design of experiment and the second six were connected with the responses of design. The numbers of carbon dioxide emitters, the number of oxygen scavengers and the Ratio were selected in a range for the goal of optimization because in the study area this is the normal condition of use. While the factor “Time” was maximize to reach the best condition to store of fruits. In this case the importance was set at 5 points to emphasize the role of this parameter respect the others.

For responses constraints, the Visual acceptability, Visual score and Global acceptability were maximize while the percentage of mouldy berries that obviously was minimized. The oxygen concentration have been constrained in the range between 2 to 21%, that represent respectively the down limit at which the berries show a damage and upper limit is the oxygen concentration in air. The “quantity” and for concentration of CO_2 was selected in range from the lowest and highest value measured in the experiment.

This conditions were summarized in Table 7.10. The Goal of optimization was combine all parameters to reach the best condition to extend the shelf life of strawberries.

Table 7.10. Determination of limit and goal of factors and the responses to optimize the RSM

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: CO_2 emitters	is in range	1	3	1	1	3
B: O_2 scavengers	is in range	1	3	1	1	3
C:Ratio	is in range	0.362	0.372	1	1	3
D:Time	maximize	6	14	1	1	5
Visual Acceptability	maximize	50	85	1	1	3
Visual Score	maximize	6	7.08	1	1	3
Global Acceptability	maximize	50	92	1	1	3
Visual Mouldy Berries	minimize	0	5	1	1	3
O_2 concentration	is in range	2	21	1	1	3
CO_2 concentration	is in range	4.26	10.3	1	1	3
Volume of CO_2	is in range	0.114	1.229	1	1	3

The models described above for each responses were used in the optimization procedure to define which is the best combination of the factors that produces the best results (response) in terms of the maintenance of quality traits and storage time. In Figure 7.18 the combination of factors to obtain the best results are presented. The combination among 2.5 carbon dioxide, about 2 oxygen

scavengers and using about 0.365 as a volume ratio can perform a shelf life of strawberries at least 12 days, determining a visual and global acceptability up to 76 % and 74 %, respectively, a 6.5 as visual score, keeping low the visual mould growth (a visual presence around 1.9%). In this condition after 12 days of storage, the head space gases concentration in the master bag will be composed by about 7.5% of oxygen and 6.2% of carbon dioxide. During this time will be expose to about $0.88\text{m}^3 \cdot \text{day CO}_2$.

The goodness of responses optimization can be defined using the satisfaction index (desirability) that evaluates the fitting between the constrains and the results of optimization. The index assumes values from 0 to 1. If the results satisfy the constrains, the value will be 1; in opposite case the value will be 0. In this study the result was 0.64 defining a good matching between constrains and the results (Figure 7.19).

Figure 7.18. Results of the numerical optimization for each variables considered.

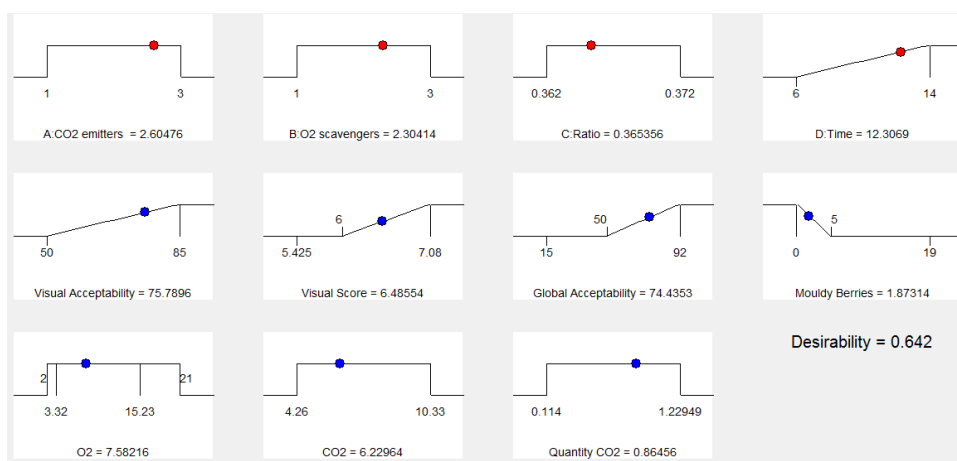
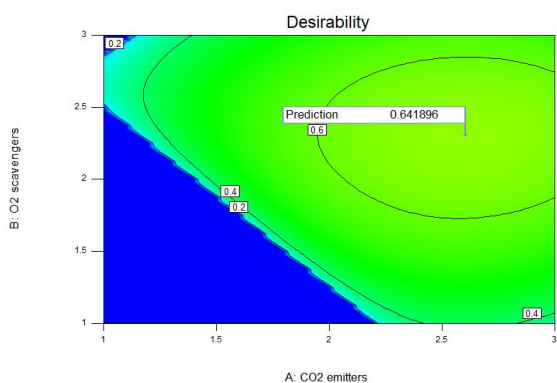


Figure 7.19. Result of the optimization in terms of the desirability of the result



7.5 CONCLUSIONS

This study investigated and measured the effectiveness of carbon dioxide emitters and oxygen scavengers to maintain the quality attributes of fresh strawberries. The use of active packaging solutions permits to reach 12 days of shelf life, definitely more than the berries stored in air without master bag solutions as reported in the previous chapter (2 days).

According to the results, the correct concentration of oxygen and carbon dioxide inside the master bag has been reached using 2.5 carbon dioxide, 2 oxygen scavengers and placing about 750 g of product inside the master bag. The easy solution proposed in this study can be applied in real conditions and verifies its efficacy to extend the shelf life.

In particular this study showed the potential of the Response Surface Methodology for understanding the evolution of the responses of the RSM and optimize the factors of packaging systems (active devices and surface-unfilled volume ratio) to reach the best conditions to prolong the shelf life of berries.

The correct packaging design can contribute not only to extend the shelf life but also to reduce the food loss thanks to the extension of the number of days in which the product can be consumed at a high quality level .

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8-COMPARATIVE LIFE CYCLE ASSESSMENT OF STRAWBERRIES STORED IN DIFFERENT PACKAGING SOLUTIONS

8.1 INTRODUCTION

Numerous studies have been focused on the environmental load of protected crops in greenhouses (Van Woerden, 2001; Williams et al., 2008) and tunnel greenhouses in the Mediterranean area (Vallejo, 2004; Romero- Gámez et al., 2009; Torrellas et al., 2012). Compared to herbaceous crops the production of fruits is commonly considered a field of low environmental impact due to the lower requirements in terms of raw materials and energy than the animal products (Granatstein and Kupferman, 2006). The plant implant has amortised for trees having long production (i.e. 15 year), while for annual crop, as strawberries, the environmental load can increase. Traditionally, environmental costs in orchards have been studied in terms of both consumption of resources (water, soil, air, energy, etc.) and impacts (pollution, risks to human health and ecosystems, reduced biodiversity, etc.; Reganold et al., 2001; Mordini et al., 2009).

As reported by FAO in 2012 the first strawberry grower in the world has been the USA with a production covering the 30% of the world market (about 1.300.000 tons). The Italian production has a small piece of this market with 110.000 tons produced in 2013 (ISTAT 2014). The production of strawberries has been still increasing but these fruits are very perishable and transport for long distance can cause a large amount of food loss. In particular, a WRAP study (2011) estimated the loss and waste in different steps of supply chain in UK country up to 30 %. The principal loss happened during harvest (2-20 %) depending on different factors such as harvest methodology and weather condition; other losses of products were 2-3 % during packaging step and 2-3% at retailer. In addition, about 2-5 % of strawberry can be wasted during the storage mainly due to the inefficiency of cold chain or logistic management. Moreover, the food waste during the consumption step of fresh fruits and vegetables up to 19% should be considered (FAO, 2011).

Packaging and its functions may play a significant role for limiting the amount of food waste in households and along supply chain. Williams et al. (2012) emphasizes the importance of learning more about how the packaging attributes can affect the food waste in households. In that study, food waste up to 25 % was related to the packaging design attributes, either those observed by consumers (i.e. easy to empty, too big packaging) or that one considered by the authors (information attribute best-before-date).

The growers and the researchers have been studying different techniques to extend the shelf life of fruit including the packaging under atmosphere modification to control the oxygen and carbon dioxide concentrations around the fruits. The atmosphere modification in packaged fruit can be obtained following two principal strategies: matching the suitable correct film permeability with the fruit respiration rate of packaged fruit at steady-state condition of oxygen and carbon dioxide (Kader et al. 1989) and establish the correct conditions to extend the shelf life of fruits by means active packaging devices. The former system is known as passive atmosphere solution. In the latter case the active devices (e.g. carbon dioxide emitters and oxygen scavengers) can be placed in the packaging in order to modify the atmosphere around the fruits quicker than the passive solution (Agar et al., 1990; Robbins and Fellman, 1993).

The use of oxygen absorbers and carbon dioxide emitters, in combination with an adequate film permeability, allows the proper concentrations of oxygen and carbon dioxide to be reached. The recommended gas partial pressures for the strawberries are in the range between 5-10 kPa for O₂ (Joles et al. 1994) and 15-20 kPa for CO₂ (Beaudry, 1999). Lower levels of oxygen can slow down the respiration and decay (Beaudry, 2000), and higher concentration of CO₂ can inhibit the fungal growth (Brown, 1992), and reduce the loss of firmness (Jacxsens et al., 2000; Day, 2001) and the respiration rate.

Using these systems (passive and active packaging) the shelf-life extension of berries can be achieved and so, an environmental load reduction can be obtained. The measurement of the possible decreasing in environmental load associated to the shelf life extension was carried out with the Life Cycle Assessment (LCA) methodology which was applied to the different packaging solutions.

8.2 MATERIALS AND METHODS

The strawberries were cultivated in Veneto region (north of Italy) and harvested at ripening. This area shows the pedoclimatic conditions supporting the production of strawberries (Agnolin, 2007).

8.2.1 Goal and scope of application

The goal of this study was to evaluate the environmental impact of production and distribution of fresh strawberries fruits packaged in different methods: “Traditional” sale unit (Base scenario, A), Passive Atmosphere Modification solution in master bag (B scenario) and Active Atmosphere Modification solution in master bag with devices (C scenario) as two alternative scenarios of the “Traditional” packaging. The characteristics of packaging solutions are as follows:

A) Lidded macro-perforated PET trays containing 250 g of berries, stored in air and considered as “traditional” packaging. Strawberries stored in this solution have a shelf life of 2 days (Chapter 6);

B) Three lidded macro-perforated PET trays containing 750 g of berries inserted into master bags (52*31 cm) made of Low Density Polyethylene (LDPE; oxygen transmission rate at 23 °C and 0 %RH equal to 4000 cc m⁻² day⁻¹, carbon dioxide transmission rate at 23 °C and 0 %RH equal to 30000 cc m⁻² day⁻¹, water vapour transmission rate at 38 °C and 90 %RH equal to 21.7 g m⁻² day⁻¹). This solution was referred to a passive modified packaging solution; Strawberries stored in this solution have a shelf life of 6 days (Chapter 6);

C) Three macro-perforated PET trays (containing 735 g of berries) inserted into a master bag unit (52*31 cm) made of LDPE (as described above). Before sealing, two oxygen scavenger (FreshPax® CR1, Multisorb Technologies Inc., Buffalo, NY, USA), and two carbon dioxide emitters (BioFresh®, Multisorb Technologies Inc., Buffalo, NY, USA; nominal capacity of 500 cm³) were placed into the master bag. Strawberries stored in this solution have a shelf life of 12 days (Chapter 7).

All the samples were stored in a cold chamber (5±1 °C; 70±5 %RH).

The methodology of Life Cycle Assessment (LCA) was applied in order to compare and evaluate the environmental load of the packaging systems taking into account the estimated shelf life of berries. The LCA is a standardized methodology used for estimating environmental burdens associated with life cycle of products or processes (ISO 14040, 2006). This methodology is considered to be effective for evaluating environmental performance in the agro-food and beverage fields (Roy et al. 2009).

In general, in LCA studies applied to the agro-sector, the functional unit (FU) was defined as mass of product (e.g. kg; Nally et al. 2011; Gan et al. 2011; Gonzalez-Garcia et al., 2012). For this study, FU was express as day of shelf life. In this way, the environmental impact was determined at one day of shelf life considering 750 g of strawberries (three sale units). Moreover for strawberry production, FU was considered as 1 hectare of orchard, while for packaging environmental impact evaluation, the FU was considered as 3 sale units (750 g of product).

This work was carried out from a “cradle to grave view”. The LCA model was carried out on two subsystems: Crop production (SS1) and post-harvest management of strawberries (SS2). **SS 1** involved the crop cultivation: the system boundary was set from the grower (that also provides for the packaging of fruits in PET trays) to the distribution center, taking into account all of the

processes (tillage, harrowing and ploughing, mulching, planting, fertilization, application of plant protection product and harvesting) required for cultivation and transport to central distribution. Concerning the crop production, all data were referred to the hypothetical 1-hectare plot and a 33 t of berries production in season production during full plant growth, according to the literature (Girgenti et al. 2014). **SS 2** involved the post-harvest management of fruits: the system boundary was set from gate of center distribution to consumer home. The transportation from the supermarket to the home of the consumers and the consumption of strawberries were not included in the LCA system. However, the disposal of the packaging material was taken into account as municipal management.

8.2.2. LCA inventory

For SS 1 the data were collected from a medium farm that produces only small fruits and in particular strawberries (90% of production).

The hypothetical hectare of orchard was set using 0.2 m as distance from two plants along rows and 0.50 m as distance from two rows and about 55000 plants have been implanted in the orchard. The data were collected through questionnaires submitted to the technical workers in the farm. The crop production was separated in three steps: Field operations, Fertilizing and Crop protection. The information collected in SS1 from farm workers was referred to the 750 g of product. The data for raw materials were selected in database Ecoinvent 3.0.

8.2.2.1 Field operation: The operations for strawberry production were defined from combing questionnaires information and literature data from the Italian average production of strawberries (Enama, 2005) for 1 hectare. These information were summarized in the Table 8.1.

Table 8.1. Principal field operations concerning the strawberry production analysis

Field operation	Consumption in diesel l/ha
Tillage, harrowing, and ploughing + sub soiling	100
Mulching	15
Planting	20
Fertilization	27
Application of plant protection product by field sprayer	63
Harvesting	40

The environmental impact was estimated taking into account the emissions in air of diesel burning using the results showed by the Argonne National Laboratory (Argonne 1996) as described in Table 8.2. For calculation the MJ from litre of diesel was considered 40 MJ/kg as energetic load and 0.832 kg/l as density.

Table 8.2. Emission factors for diesel fuel combustion in a farming diesel tractor

Emission factors (g/MJ diesel fuel burned)							
Hydrocarbons	CO	NO _x	PM ₁₀	SO ₂	CH ₄	N ₂ O	CO ₂
0.085	0.32	0.89	0.041	0.12	0.0042	0.0019	75.5

For greenhouses structure, the data were obtained from the questionnaires submitted to the grower and summarized in the Table 8.3. The life span of the greenhouse was estimated at 15 years according to the European Committee for Standardization (CEN 2001). For the metal structure, the steel production (unalloyed) was considered in the LCA inventory, the quantity was related to the

lifetime previously defined. For covering of greenhouse the Low Density Polyethylene (LDPE) film was used and 5 years correspond to their lifetime which was longer than that one used in other study (Torrellas et al., 2012). For irrigation in the field, the sprinkler drip systems were used to increase the efficiency of irrigation; the material of the tubing was polyvinylchloride (PVC). For the mulching a thick layer made by LDPE was used.

Table 8.3. Structure processes included in the inventory. Values are total amount of material per hectare: the life span was not considered.

Materials	Quantity (Unit)
Metal steel – unalloyed-	19500 kg/ha
LDPE – Film for covering the greenhouse-	1200 kg/ha
PVC – Tubing and piping-	100 kg/ha
LDPE – Film for mulching	620 kg/ha

Concerning the irrigation, the water was also estimated to maintain the production and humidity in the soil. The frequency of irrigation was established in twice a week for 16 weeks and 6 hours for each event. The flow in the tubing was calculated in 1 l/h. The result of these assumptions lead to 1056 m³ of waters to produce about 33000 kg of berries. The energy utilized was set in 44.2 kWh/ha (Girgenti et al., 2014).

For the fruit production, the fertilization and the plant protection treatments were applied. The base fertilization was applied as manure in order to improve the organic compounds in the soil, the quantity added every year was estimated in 50 tons per hectare. For the mineral fertilization, ammonium nitrate was used as 95 kg per hectare, single superphosphate as 70 kg per hectare, and potassium sulphate as 75 kg per hectare. Concerning the fertilization, the information about nutrient (nitrogen) removal from fruits was taken from literature. Emissions due to the fertilizer application was also included in the inventory. Nitrogen emissions (nitrate, ammonia and nitrous oxide) were modelled following the study reported by Brentrup et al. (2000). Phosphate emissions were calculated in accordance to Smil (2000), the losses of P was equal to 1% of the total applied phosphorus. This calculation was carried out using the EFE-So software (Version 2.0.0.1) and the values were reported in the Table 8.4. The emissions were reported in Table 8.5 as resulted by the EFE-So software.

Table 8.4. Conditions and values used in the EFE-So software to calculate the emission from fertilizers.

Solid Manure	Quantity/ Characteristics	References
N content in applied dose (kg/ha)	250	IPCC 2006
Ammonia content in applied dose (kg/ha)	25	IPCC 2006
Time between application and precipitation or incorporation (h)	4	Farmer questionnaire
Temperature (°C)	5-10	Farmer questionnaire
Precipitation (mm)	0-2	Farmer questionnaire
Ammonium nitrate		
N content in applied dose (kg/ha)	95	IPCC 2006
European country		Italy
Nitrogen deposition (kg/ha)	18.9	Sheffield et al., 2006
Yield (t/ha)	33	Farmer questionnaire
Nitrogen content in crop harvest (kgN/t)	1.067	Giampieri et al., 2012
Other output (t/ha)	0.3	Farmer questionnaire
Nitrogen content in other output (kgN/t)	27.6	Jurik et al., 1982
Soil type	Sandy loam	Farmer questionnaire
Summer precipitation (mm)	194	Frontero, 2010
Winter precipitation (mm)	270	Frontero, 2010

Table 8.5. Results of the EFE-So software: emissions of fertilizers into water and soil.

Output	Compound	Quantity	Unit
Emission to air			
	Dinitrogen monoxide	3.96	kg
	Ammonia	28.44	kg
Emission to water			
	Nitrate	163.3	kg
	Phosphate	0.14	kg

In this study pesticides were selected in the database Ecoinvent 3.0: the “pesticide unspecific” made in Europe and, for potassium carbonate, the global production was used.

Pesticides used in agriculture can cause undesirable effects on humans and the natural environment. One of the objectives of integrated agriculture is the elimination or reduction of possible sources of environmental pollution such as pesticides. To achieve this objective, farmers need a method to

assist them in estimating the environmental impact of pesticide use (Van der Werf, 1996). The LCA methodology can define the environmental impact of pesticides used in the agricultural. For this study the type and quantity of pesticides used in this crop production are summarized in Table 8.6.

Emission of pesticides into the air was calculated on the basis of the research carried out by Van den Berg et al. (1999). These authors indicated that 30–50 % of total sprayed pesticides were emitted into the air due to spray drift and volatilization.

This study adopted 40 % to calculate the emission from pesticides into the air, considering the crop type and growing method used. This percentage balanced two considerations. Firstly, some of the pesticides into the air end up on the greenhouse linings in which the plants are cultivated, which reduces the emission to the (outside) air. Secondly, the high dose of chemicals applied and the hotter weather conditions in the greenhouse increases the emission of pesticides into the air. The pesticides fall down into the soil for 15 % and the runoff into the water was estimate in the 10 % of the pesticides presented into the soil (Sahle and Potting; 2013). The emissions were summarized into Table 8.7.

Table 8.6. Principal compound used as pesticides in strawberries production

Operation	Compound	Quantity	Unit
Plant protection treatments	Abamectin	1.2	kg/ha/year
	Cyprodinil+Fludioxinil	1.6	kg/ha/year
	Potassium bicarbonate	10	kg/ha/year
	Fenitrotrion	1.5	kg/ha/year

Table 8.7. Emissions into air, soil and water of pesticides used in strawberries production for 1 ha; the values were expressed in kg

Compounds	Air	Soil	Water
Abamectin	0.48	0.18	0.018
Cyprodinil	0.32	0.12	0.012
Fludioxinil	0.32	0.12	0.012
Potassium bicarbonate	4	1.5	0.15

8.2.2.2 Transport

Concerning the transportation of trays, the distance from packaging producer industry (located in Emilia Romagna region) to the producer was 220 km. It was assumed that all the transportations involved a full load trucks. Master bag -film low density polyethylene (LDPE) 25 µm- was transported between local company and the central of distribution (60 km).

For the transport of fruit in the trays from grower to distributional center located in Milan, commercial truck (32 t) was considered (Euro 3). The distance was calculated by Web software (Google Maps, Google inc., Parkway Mountain View, CA) and it was about 240 km. It is assumed that all the transportations involved a full load trucks.

The second SubSystem (SS2) involves the post-harvest management of fruits. The fruits were stored in a cold chamber to maintain the quality of products. In a distributional center the

strawberries were delivered to retailer as function of their requests while in the alternative scenarios, in distributional center, the fruits were packed in master bags before storage.

8.2.2.3 Refrigeration

The second SubSystem (SS2) involved the post-harvest management of fruits. The fruits were stored in a cold chamber to maintain their quality for longer times. In a distributional center the strawberries were delivered to retailer as function of their requests, while, in the alternative scenarios, the fruits could be packed in master bags before storage to extend their shelf life at distributional center.

The products were refrigerated from field temperature, estimated, in the spring-summer condition at 25°C to reach the storage temperature closed 4°C by cooler. In literature the optimal temperature to store the strawberries is -0.5 - 0°C (Cantwell, 2002) but in traditional chamber the fruits were refrigerated from 2 to 4°C (Nunes et al. 2009).

A computational approach was performed to estimate the electrical energy required for cooling the strawberries and to maintain the temperature during the storage (Bonauguri and Miari, 1988). The calculation took into account different heat sources presented in the cooler system: the air inside the refrigerated chamber and the air exchange due to door opening during the fruits movement, the heat coming from walls, ceiling and floor, (considering 25 °C as external temperature) and from fruit metabolism (0.08 W/kg; Sharma et al., 2013), the energy utilized by lights and other devices used inside the cooler. The calculation was determined 4.63 Wh per kg of product.

8.2.2.4 Packaging components

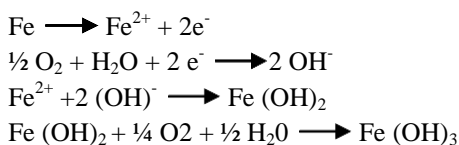
8.2.2.4.1 Active devices

Oxygen scavenger

To estimate the environmental burden of active devices it was used the common formulation found in literature, because the recipe is covered by industrial secret. The oxygen scavengers are self-activate devices and their functionality is performed through the oxidation reaction presented in Equation 8.1 (Schroeder et al. 2001). Usually, the well-known coformulants were the silica gel and sodium chloride (Brody et al., 1995).

The high density polyethylene (HDPE) is the film where these compounds are contained (0.49g).

The iron powder to scavenge 100 cc of O₂ (maximum scavenging for this device, CR1 as reported in M&M) was 0.33 g and 0.162 g of pure water.

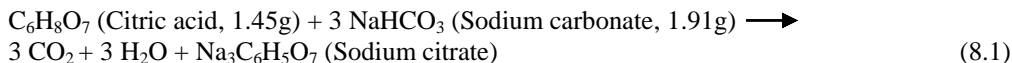


For the silica gel, the absorption of water was estimated at 15 % (w/w) that correspond at 50 % of their maximum absorption value equal to 30 % (Afonsoa & Silveira, 2005). To absorb the 0.162g of pure water (used in the reaction 1) 1.08 g of silica gel were required.

As well known in the oxygen scavenger the chlorine ion was required as chemical catalyzer of the oxidation reaction: the sodium chloride added was 1.6 g.

Carbon dioxide emitter

For Carbon Dioxide Emitters, the reaction presented equation was used to determine the weight of each ingredient.



8.2.2.4.2 Tray

The tray was made by polyethilenterefalate (PET) and in the inventory analysis 13 g of the raw materials (Europe) were considered for its production and the thermoforming energy requirement was defined as 1.12 Wh for each tray.

8.2.2.4.3 Master bag film

The film was made by low density polyethylene and in the inventory 7.72g of film were inserted as quantity, with an efficiency of production from raw material (pellet) to film up to 97.6%. The extrusion energy requirement was defined as 2 kWh/kg of product.

8.2.2.5 End-life

In the end-life step only the packaging disposal was considered. The Lombardy was considered a region where the packaging waste was collected.

Plastic collection

As Grosso et al. (2012) described, the plastic collection is made in two different ways: kerbside collection in the 33% of collection cases while in the remaining cases (67%) using waste containers on road.

In kerbside collection, the management of characteristics is explained in Table 8.8.

Table 8.8. The management kerbside collection

Transporter	Van < 3.5 t	Lorry 16-32 t
Percentage of used in collection	59.4	40.6
Distance	48.8	48.8

Plastic recycle

Before recycling, the plastics have to be selected to remove the undesirable items and unrecyclable plastics. This phase requires per 1 ton of plastic about 26.6 kWh of electricity and 84 MJ of diesel (Grosso et al., 2012). The efficiency of plastic selection system was assumed as 100% due to the high purity of plastic material.

For PET tray, a 95% of recycling was assumed as efficiency of systems and the remaining 5% was collected in the municipal waste. The whole impact generated for PET production was considered as an avoided impact for the system (Levi et al. 2011).

For LDPE bag, a 95% of recycling was assumed as efficiency of systems and the remaining 5% was collected in the municipal waste. In this case, the study described by Rigamonti and Grosso (2009) was used to model the energy and material necessary for recycling the LDPE (Table 8.9).

Table 8.9. Consumption of energy and raw materials used in recycle process of LPDE.

Input for 1 ton of LDPE	Quantity
Electricity for recycle	381 kWh
Electricity for produce the rod	200 kWh
Natural gas	650 MJ
Water	1.78 m ³

8.2.3 Impact assessment

The software SimaPro® 8.0.1 (PRé Consultants bv. Netherlands) was used for the computational of the inventories data. Among the steps defined within the LCA, only classification and characterization stages were undertaken (ISO, 14040, 2006). According to other studies concerning the agricultural-packaging systems ReCiPe Midpoint (H) V1.08 - Europe Recipe H model was used and the following categories were selected to evaluate the environmental load of strawberries supply chain: Climate change, Ozone depletion, Terrestrial acidification, Freshwater eutrophication, Marine eutrophication, Human toxicity and Fossil depletion (Table 8.10).

Table 8.10. Impact categories considered in the analysis according to the ReCiPe Midpoint (H) V1.08 - Europe Recipe (Hierarchy) method.

Impact category	Unit
Climate change	kg CO₂ eq
Ozone depletion	kg CFC-11 eq
Terrestrial acidification	kg SO₂ eq
Freshwater eutrophication	kg P eq
Marine eutrophication	kg N eq
Human toxicity	kg 1,4-DB eq
Fossil depletion	kg oil eq

8.3 RESULTS AND DISCUSSION

8.3.1 Production

Many authors agree that the food production is the main factor in the environment load along the supply chain (Peano et al. 2015; Girgenti et al. 2014; and Seppala et al. 2009, Roy et al. 2009). Figure 8.1 shows the results concerning to the environmental load of the strawberries production. In the figure, the impacts of 750 g of strawberries (3 trays) can be share in the different crop production phases mentioned as above related to the fruit growth. For each impact categories, it is possible to define the main steps mainly affecting the environmental burden. For climate change, the greenhouse structure (30 %) and the film for mulching (25 %) created a large amount of the greenhouse gases. The ozone layer decreased its thickness due to the substances emitted for production the greenhouse structure (23 %) and the pesticides emissions (20 %). In the Terrestrial acidification and the Marine eutrophication, the fertilizers used to cultivate the strawberries lead to the highest role on environmental impact up to 74 % and 97 %, respectively for Terrestrial acidification and the Marine eutrophication. This is probably due to the run off the nutrients from soil to water (Muñoz et al. 2010) and emission in air of nitrogen and sulphur compounds. For freshwater eutrophication, the most important step was the material of greenhouse due to the substances used in the metal production process (50 %). The greenhouse structure showed an high load in different impact categories as reported in literature (Torrellas et al., 2012).

The plant protection compounds generated not only an environmental damage but also a Human toxicity due the hazard molecules used against the pest and fungi. For this category, the plant protection compounds generate the highest impact, about 38 %.

For covering the crop and protect it against the weeds in the farm the LDPE film was used, and its production needs to use the Fossil compounds as oil reducing the availability of this substance.

In Table 8.11 was reported the values of environmental load for different impact categories associated to different steps of strawberry production. As mentioned above, for each impact category the most important factor on the environmental load was defined. The Life Cycle Analysis can contribute to improve the knowledge about the development of more performing fruit production systems where it is possible to reduce the loss of nutrient, for example using fertirrigation system or organic fertilizer. The mulching film material could be replaced with other materials having lower environmental load, for example using Bio-based material as suggested by Girgenti et al. (2014).

The total amount of carbon dioxide emitted for production of strawberries agreed with life cycle assessment of production in Spain of strawberries (0.35 kg CO₂ eq/kg Williams et al., 2008) and the production developed in the north of Italy (0.053 kg CO₂ eq/250g of berries; Girgenti et al., 2013).

Figure 8.1. Impacts of the production subsystems on the environmental load expressed as relative contribute

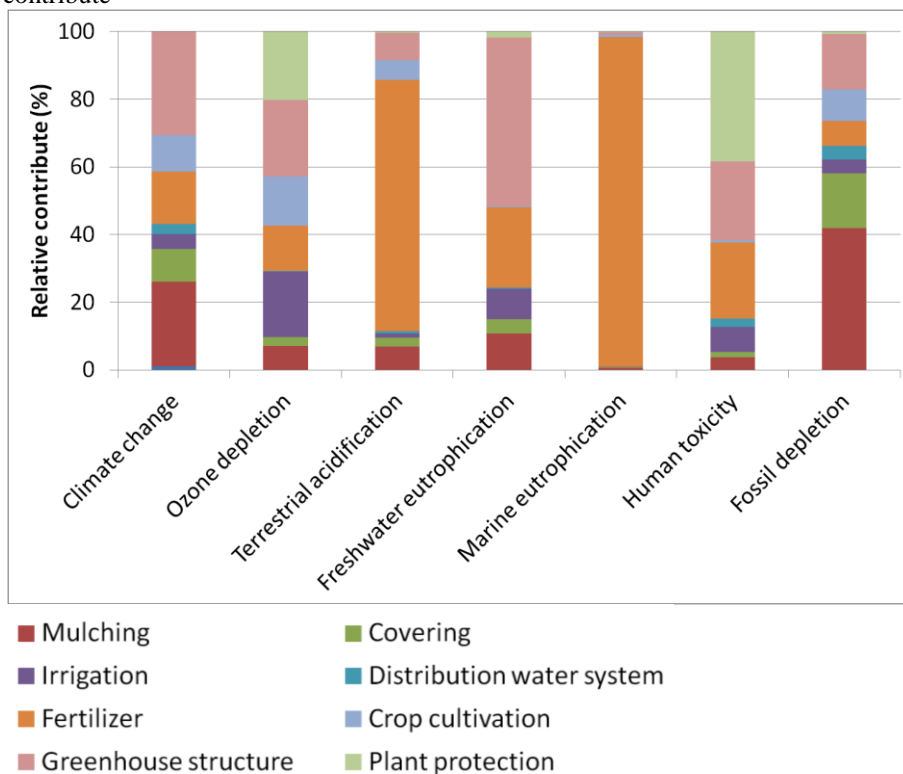


Table 8.11. Environmental results for the strawberries production for each impact category

Impact category	Unit	Total	Mulching	Covering	Irrigation	Distribution water system
Climate change	kg CO ₂ eq	1.6*10 ⁻¹	4.1*10 ⁻²	1.6*10 ⁻²	7.2*10 ⁻⁴	4.8*10 ⁻³
Ozone depletion	kg CFC-11 eq	6.3*10 ⁻⁹	5.3*10 ⁻¹⁰	2.0*10 ⁻¹⁰	1.5*10 ⁻¹⁰	1.8*10 ⁻¹¹
Terrestrial acidification	kg SO ₂ eq	2.3*10 ⁻³	1.6*10 ⁻⁴	6.2*10 ⁻⁵	3.1*10 ⁻⁶	1.3*10 ⁻⁵
Freshwater eutrophication	kg P eq	3.6*10 ⁻⁵	4.2*10 ⁻⁶	1.6*10 ⁻⁶	3.4*10 ⁻⁷	1.6*10 ⁻⁷
Marine eutrophication	kg N eq	9.5*10 ⁻⁴	4.4*10 ⁻⁶	1.7*10 ⁻⁶	1.7*10 ⁻⁷	8.2*10 ⁻⁷
Human toxicity	kg 1,4-DB eq	1.3*10 ⁻²	5.4*10 ⁻⁴	2.1*10 ⁻⁴	1.1*10 ⁻⁴	3.6*10 ⁻⁴
Fossil depletion	kg oil eq	5.8*10 ⁻²	2.5*10 ⁻²	9.8*10 ⁻³	2.4*10 ⁻⁴	2.5*10 ⁻³

8.3.2 Packaging

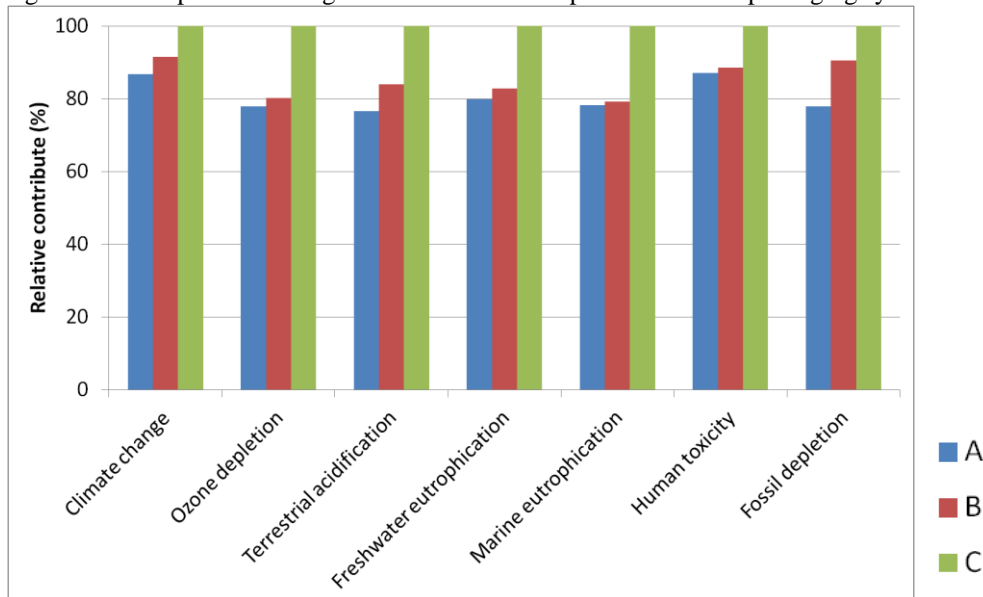
As expected, the analysis of packaging systems demonstrated the increase of the environmental burn due to the material and the active compounds used in the active packaging systems (Table 8.12).

The absolute amount of the different impacts was negligible among the three packaging systems, but in relative graph the small difference generated an important difference in particular in ozone depletion, terrestrial acidification, marine and freshwater eutrophication (Figure 8.2).

Table 8.12. Environmental results for different packaging solutions

Impact category	Unit	“Traditional” package	Passive package	Active package
Climate change	kg CO ₂ eq	2.17*10 ⁻¹	2.29*10 ⁻¹	2.5*10 ⁻¹
Ozone depletion	kg CFC-11 eq	5.22*10 ⁻⁹	5.38*10 ⁻⁹	6.70*10 ⁻⁹
Terrestrial acidification	kg SO ₂ eq	4.92*10 ⁻⁴	5.39*10 ⁻⁴	6.42*10 ⁻⁴
Freshwater eutrophication	kg P eq	3.30*10 ⁻⁵	3.43*10 ⁻⁵	4.14*10 ⁻⁵
Marine eutrophication	kg N eq	1.12*10 ⁻⁴	1.13*10 ⁻⁴	1.43*10 ⁻⁴
Human toxicity	kg 1,4-DB eq	1.8*10 ⁻²	2.1*10 ⁻³	3.12*10 ⁻³
Fossil depletion	kg oil eq	4.64*10 ⁻²	5.38*10 ⁻²	5.95*10 ⁻²

Figure 8.2. Comparison among the environmental impact of different packaging systems



8.3.3 Environmental daily load of packaging solution

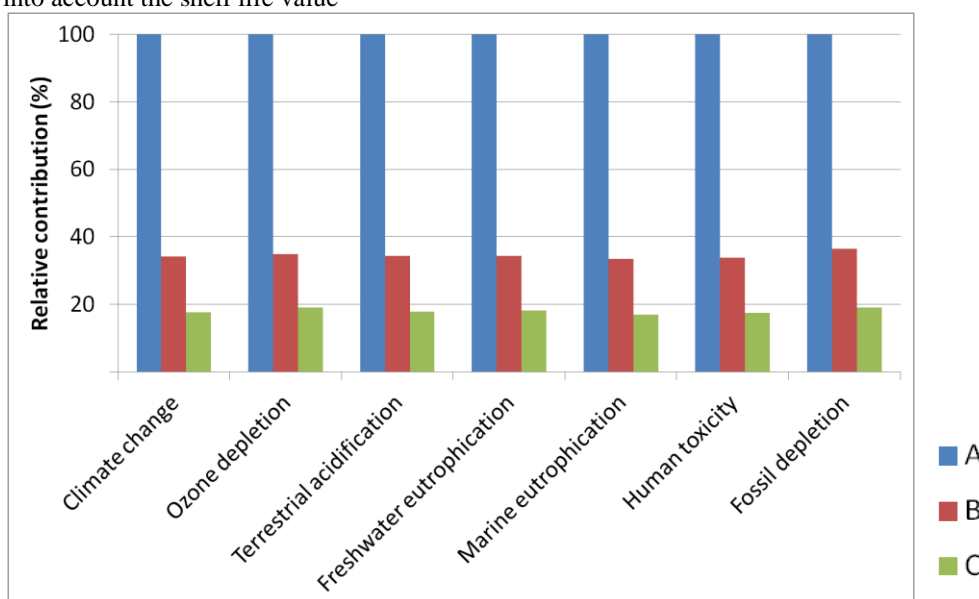
The choice of using the day of shelf life as the functional unit means that the environmental impacts were shared along the lifetime. In this way, a “daily” impact can be defined for each packaging solution adopted.

For each impact category, the differences between base scenarios and alternative scenarios were evaluated. The packaging solution A had only 2 days of shelf life and this condition determined the highest daily impact among the packaging solutions studied. Whereas the packaging solution B and C determined a significative reduction in terms of environmental load around 66 % and 82 %, respectively (Figure 8.3). As assumed by some authors (Williams et al. 2010, Roy et al. 2009, FAO, 2011), a correct packaging can contribute to reduce the overall impact of the system linked also the reduction of food waste (Almenar et al, 2010). The differences among packaging systems for each impact categories were ascribable to the relative high impact of the active compounds or the master bag film in the estimation of the environmental impact. The values for each impact categories showed a big differences when food product was took into account (Table 8.13). The packaging systems differences among the three scenarios were minimized due to the biggest weight of food production, in terms of the environmental load, on entire impact. This means that the packaging environmental load could be improved to reduce the entire environmental load (Williams et al. 2011).

Table 8.13. The percentage of environmental load of different packaging solutions

Impact category	Unit	“Traditional” solution	Passive solution	Active solution
Climate change	kg CO ₂ eq	$5.80 \cdot 10^{-1}$	$1.98 \cdot 10^{-1}$	$1.02 \cdot 10^{-1}$
Ozone depletion	kg CFC-11 eq	$1.46 \cdot 10^{-8}$	$5.09 \cdot 10^{-9}$	$2.77 \cdot 10^{-9}$
Terrestrial acidification	kg SO ₂ eq	$1.98 \cdot 10^{-3}$	$6.82 \cdot 10^{-4}$	$3.25 \cdot 10^{-4}$
Freshwater eutrophication	kg P eq	$7.14 \cdot 10^{-5}$	$2.45 \cdot 10^{-5}$	$1.29 \cdot 10^{-5}$
Marine eutrophication	kg N eq	$7.9 \cdot 10^{-4}$	$2.64 \cdot 10^{-4}$	$1.34 \cdot 10^{-4}$
Human toxicity	kg 1,4-DB eq	$2.3 \cdot 10^{-2}$	$7.77 \cdot 10^{-3}$	$4.02 \cdot 10^{-3}$
Fossil depletion	kg oil eq	$8.7 \cdot 10^{-2}$	$3.18 \cdot 10^{-2}$	$1.66 \cdot 10^{-2}$

Figure 8.3. Comparison among the environmental impact of different packaging solutions taking into account the shelf life value



8.4 CONCLUSION

The shelf life values estimated in this study allowed a clear definition of the environmental sustainability of the packaging systems due to the reduction of environmental impact correlated to the increase in shelf life.

As a result of this study, the environmental impact was assessed for the strawberries stored in different packaging solutions identifying the best option in terms of lower environmental burn. The LCA methodology also permitted to define the best solution for strawberry storage in terms of the lowest environmental impact: with the active packaging solution the reduction of the environmental load can be reached with a consequent reduction in food chain.

Over the last years, the interest in the environmental impacts associated with food systems has been strongly growing. Some studies have confirmed the relative importance of “food and beverages

consumption” in contributing to environmental impacts (Bacenetti et al. 2015; Williams et al 2011). Within the food chain, also the waste management contributes to the overall environmental burden of food products (FAO, 2013). Among the different mitigation strategies, some studies highlighted that the optimization of packaging solution can be an effective solution to decrease the environmental load of the food systems (Piergiovanni et al., 2014; Williams et al 2011). Future researches need to better specify the effect of the extension shelf life of this fresh product on the supply chain and the consumer habits. The knowledge concerning the shelf life extension should be improved as a response of sustainability in terms of environmental load, social and economic effect. One of the goal imposed by FAO in contrasting the fame is the reduction of the global food waste as a contribution to feeding nine billion people by 2050. The limitation of this virtuoso process is the lack of knowledge about the causes and the reticence of the authorities and the politicians (Parfitt et al., 2010).

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CONFERENCE ABSTRACTS

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Integrated approaches to assess the shelf-life and support sustainability strategies of packaged foods

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The aim of this PhD research project is double: *a*) to develop new protocols to compute the shelf-life dating of representative foodstuffs which are characterized by novelties in packaging technologies and environmental exposition and *b*) to define innovative indicators that could be useful to implement the traditional LCA approaches for the estimation of a packaged food sustainability along the supply chain.

Approcci integrati per la valutazione della *shelf-life* di alimenti confezionati a supporto di strategie di sostenibilità

Lo scopo del progetto di dottorato è duplice: *a*) sviluppare nuovi protocolli per la valutazione della *shelf-life* di alimenti confezionati, *b*) definire innovativi indicatori che possono essere usati per implementare il tradizionale approccio LCA al fine di stimare il peso di modifiche della *shelf-life* sulla sostenibilità lungo l'intera filiera di produzione e distribuzione.

1. State of Arts

All foods are susceptible to quality and safety losses. The shelf-life can be defined as a finite length of time after production and packaging during which the food product retains a required level of quality under well-defined storage conditions; therefore, shelf-life should reflect only the quality loss dynamics (Nicoli, 2012). The definition of shelf-life requires a multidisciplinary approach because different driving forces are involved: regulatory, economics, marketing, social. From the beginning of shelf-life studies many scientific works in literature focused the attention on this value in packaged food products; unfortunately, few papers only assessed the shelf-life in a correct way determining the limit of quality where product was failing.

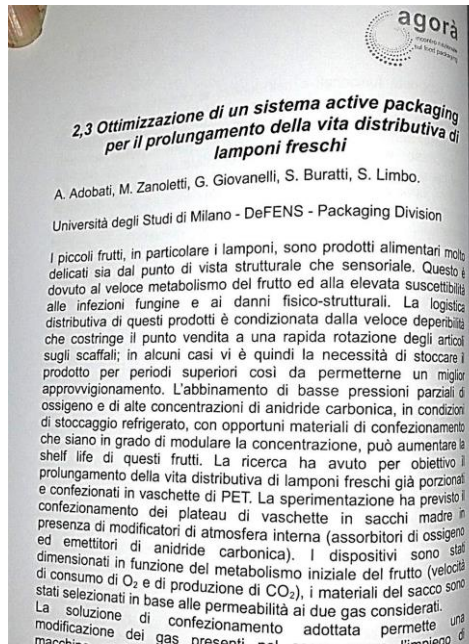
The first step in assessing the shelf-life is to identify the main parameter that describes the food quality decay of packaged food during storage time. It is possible to use traditional techniques (e.g. chemical analyses, physical analyses, microbiological indexes or sensory attributes) or to exploit otherwise an innovative procedure gathering different decay-parameters as multivariate analysis (Pedro and Ferreira, 2006).

The second step is to set the acceptability limit determining the value (or the range of values) that discriminate whether one product is acceptable or not by consumers, otherwise defined as the point where all decay reactions reach their maximum acceleration (Limbo et al., 2009). Usually industry and researchers do not specify this depletion index in a measurable way; its determination is complicated and in many papers this index is not evaluated or is arbitrary set.

The last step in shelf-life assessment is to monitor the critical indicator under real-time or accelerated conditions of storage to evaluate quality changes. Data collected during testing are modeled to obtain parameters able to describe the kinetics behaviors, thus to predict the shelf-life once the acceptability limit has been defined. These models have to take into account the following effects on overall storage: *a*) environmental factors like gas concentration, moisture, light intensity and temperature; *b*) packaging factors like gas permeability of plastic materials; *c*) food or process factor like ingredients or thermal treatments. In this sense few strategies have been outlined, especially when more than one accelerating variable or when accelerating factors different from temperature are used to speed up the quality decay during experiment.

From this point of view there is a need of defining a protocol to identify the correct shelf-life and to develop a sustainability-trend. Shelf-life studies are expensive for food companies but methods to define the date of fail has to be improved. The potential inaccuracies in its definition may be responsible of two different scenarios: a shelf-life overestimation that could cause consumer complaints, product recalls, ineffective logistic impacts, food losses, etc... and a shelf-life underestimation that could cause serious, expensive food losses and wastes, obstructing the optimization of formulation, processing and packaging solutions, therefore the potentiality for a shelf-life extension. Techniques such as Life Cycle Assessment (LCA) can be used to quantify environmental impact of the food losses and waste arising from an incorrect definition of shelf-life. This approach is in accord with a recent study (Wikström & Williams, 2010) which stressed the importance of increasing the knowledge about the amount of food losses, the environmental impact due to losses and the reasons why losses arise. In fact

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SHELF LIFE EXTENSION OF SMALL FRUITS: PASSIVE AND ACTIVE MODIFIED ATMOSPHERE INSIDE MASTER BAG SOLUTIONS

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The second year activities of this PhD project focus on the shelf life assessment of small red fruits (strawberry and raspberry). These products are non-climacteric highly perishable fruits because susceptible to mechanical injury during picking and transportation. Water loss, mould growth and off-flavour production during storage limit their shelf life to few days. In particular, raspberries have a high added value and high nutritional value, but unfortunately the mass loss during distribution and domestic storage has been estimated around 50%. The adoption of a suitable packaging material and/or technology could prolong the shelf life, reducing the waste along the supply chain.

Estensione della vita di scaffale di piccoli frutti: utilizzo di atmosfere modificate attive e passive all'interno di sacchi madre

La prima attività del progetto di dottorato descrive la valutazione della shelf life in prodotti freschi come piccoli frutti (fragole e lamponi). Questi prodotti sono non-climaterici, altamente deteriorabili perché suscettibili ai danni meccanici durante il trasporto e la raccolta. Inoltre la perdita di peso, la crescita di muffe e gli odori sgradevoli durante la conservazione limitano la loro vita di scaffale a pochi giorni (meno di una settimana). In particolare modo per i lamponi, si stimano perdite fino al 50% durante la distribuzione e lo stoccaggio domestico. L'adozione di un materiale di confezionamento adeguato e/o di una opportuna tecnologia di packaging può contribuire al prolungamento della shelf life e, quindi, alla riduzione delle perdite di questi frutti lungo la supply chain.

Key words: shelf life extension, small fruits, master bag, active and passive atmosphere.

1. Introduction

The general purpose of this study was to investigate the ability of passive and active atmosphere in extending the shelf life of red raspberries. During storage, the fast decay of quality could be controlled by means of packaging technologies able to modify the gas composition inside the package, slowing down the fruit metabolism and the microorganisms growth. The modification of atmosphere can be achieved through the passive or active solutions. In the first case, the permeability of the packaging material and its selectivity towards gas can be combined with the fruit metabolism to control the equilibrium between fruit respiration and head space composition. In the second case, it is possible to introduce an active device that changes the gas composition inside the packaging, releasing or absorbing oxygen and/or carbon dioxide.

2. Materials and Methods

Fruits

Red Raspberries (*Rubus ideasu L.*) cv. Erika (ripening period July - August) were provided by a local supermarket in Milan; the berries were picked at commercial ripening stage, packaged in lidded PET macro-perforated trays (125g) and transported to the laboratory as quickly as possible. The fruits were immediately stored in a cold chamber, after their packaging in different solutions.

1) Passive atmosphere: packaging and storage

For each trial, two PET trays (125g of berries) were packed using a master bag solution, made of plastic materials with different permeability to oxygen and carbon dioxide (high, medium and low barrier), as reported in Table 1. The control sample consisted in PET shell claim: this package does not provide any gas barrier effect due to the presence of macro-holes. The following analyses were carried out: Damaged berries, both physically damaged and mouldy berries (%); Weight loss, (%); Colour (CIE L*, a* and b* parameters); total solids (g/100g); soluble solids (g/100g); pH ; Titratable acidity (g citric acid/100 g); consistency by single compression test (energy at 60% deformation). For each test trial, the sampling and analyses were performed at time 0 and after 2,4 and 7 days of storage in a cold dark chamber (5±1°C).

4. **Adobati A.** (2015). Active Packaging in master bag solutions and Shelf Life Extension of red Raspberries and Strawberries: a reliable strategy to reduce Food Loss. 20th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Perugia, Perugia, September 23rd-25th, 2015. (*Oral Presentation*).

**Active Packaging in master bag solutions
and Shelf Life Extension of red Raspberries and Strawberries:
a reliable strategy to reduce Food Loss**

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The PhD project focuses on the shelf life assessment of strawberries and raspberries and aims to find new packaging solutions based on master bag and active packaging to extend their shelf life. The methodology used in this study allowed the knowledge of quantitative information on Shelf Life Extension conferred by active packaging. Moreover, the Environmental Impacts of these packaging solutions, calculated by means of the Life Cycle Assessment, have been taken into account considering the benefits of the Food Loss reduction derived from the actual Shelf Life Extension.

Imballaggio attivo in soluzioni master bag ed estensione della Shelf Life: una strategia affidabile per la riduzione delle perdite alimentari

Il progetto di dottorato ha come obiettivo generale quello di stimare la vita di scaffale di fragole e lamponi e di trovare nuove soluzioni di confezionamento basate sull'impiego di master bag e di dispositivi attivi per prolungarne la vita. L'approccio metodologico utilizzato ha consentito di ricavare informazioni quantitative circa l'estensione della shelf life apportata grazie all'impiego di soluzioni di imballaggio attivo. Inoltre, l'impatto ambientale delle soluzioni a confronto, calcolato grazie alla "Life Cycle Assessment", è stato stimato considerando i benefici che la reale estensione della shelf life apporta alla riduzione delle perdite di questi alimenti.

Key words: Raspberries; Strawberries; Shelf Life Extension; Active Packaging; LCA; Food Loss

1. Introduction

A rough estimation for avoidable losses in the European Union (EU) is 280 kg per capita per year, of which 13% would arise from agricultural production, 31% from product processing and 45% from households (Beretta et al., 2013). The role of packaging in protecting fresh and processed foods is well known and documented but little research is available about the relations existing among new packaging solutions, shelf life extension and Food Loss, and waste reduction at the different levels of the supply chain. Techniques such as Life Cycle Assessment (LCA) have been largely used to quantify Environmental Impact of food production, processing and packaging operations or materials but a new assessment taking into account the food and its packaging as a whole system and incorporating the effect of Food Loss reduction is nowadays necessary. In fact, from a life cycle perspective, any assessment of the Environmental Impact of food packaging must take into account the positive benefits of reduced Food Losses in the value chain (McMillin, 2008).

In this PhD project, the possibility of extending the shelf life of red raspberries (*Rubus idaeus L.*) and strawberries (*Fragaria x Ananassa Duch.*) using active packaging solutions has been investigated, after the definition of critical indicators and cut-off criteria useful in pointing out the time at which the lifetime ends. The final aim was to estimate the role of a new packaging technology in reducing Environmental Impact along the supply chain but taking into account the benefits of the Food Loss reduction derived from the actual Shelf Life Extension. Raspberries and strawberries have a very short shelf life due to the physiological aspects such as high respiration rate, loss of firmness, susceptibility to mould and breaking down tissues. Currently, the high perishable characteristics of these fruits contribute to Food Loss along the supply chain (up to 75% until retailer, WRAP, 2011). The study on active packaging compared with traditional and passive atmosphere solutions from a whole perspective could really contribute in improving the active packaging technologies and making more efficient the food supply chain.

2. Materials and Methods

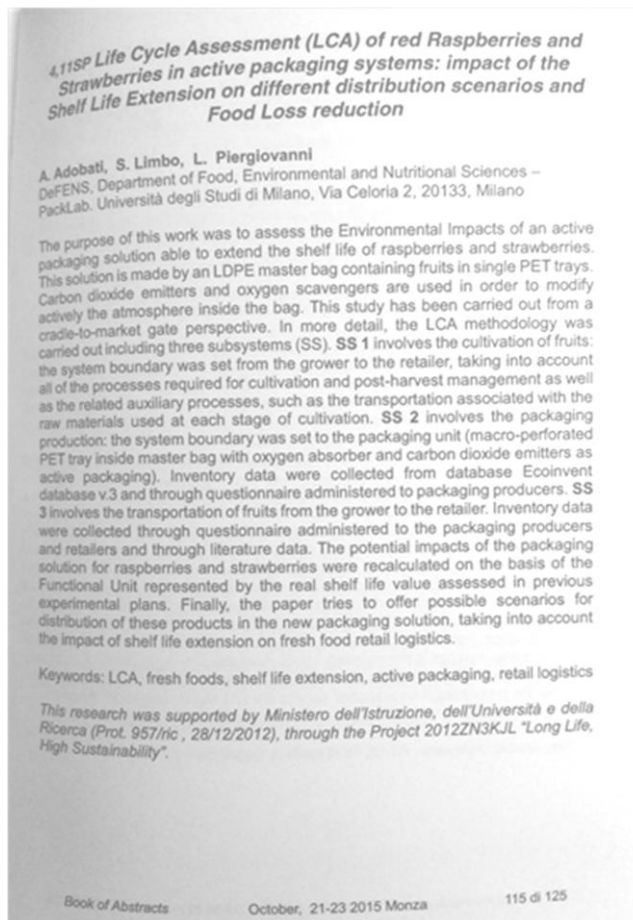
2.1 Raspberries trial

In this study Red Raspberries (*Rubus idaeus L.*) cv. Erika were used. Fruits were hand-harvested at commercial ripening stage and were packaged within 24 h; only berries with comparable colour and absence of defects and mould were selected.

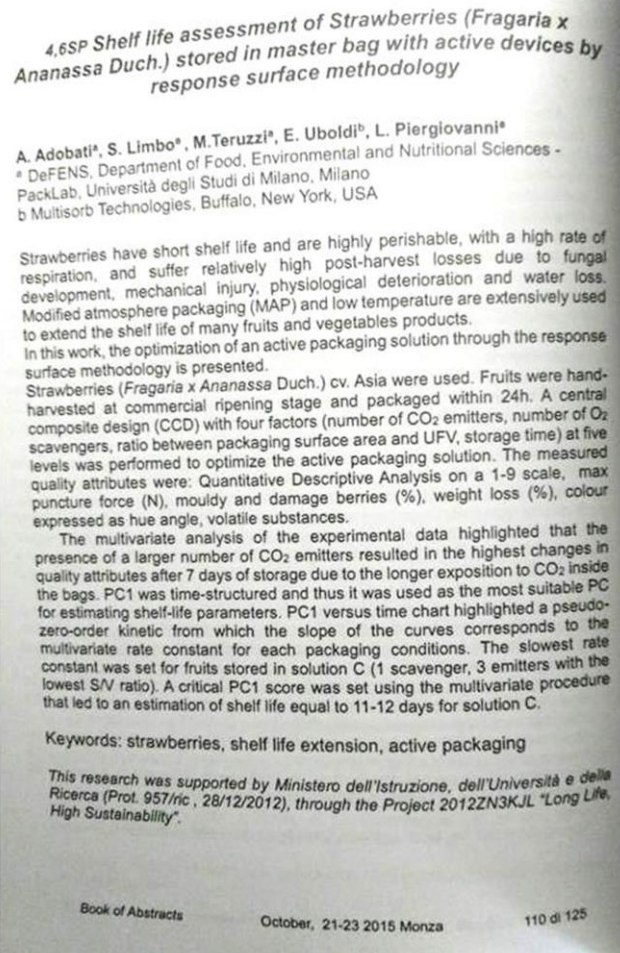
Packaging solutions

A) Lidded macro-perforated PET trays containing 125 g of berries, stored in air and considered as "traditional" packaging; B) Two lidded macro-perforated PET trays containing 125 g of berries inserted into master bags (34cm*25.5cm) made of plastic materials with different permeabilities to gas and water vapour. This solution

- 5 **Adobati A., Limbo S., Piergiovanni L.** 2015. Life Cycle Assessment (LCA) of red Raspberries and Strawberries in active packaging systems: impact of the Shelf Life Extension on different distribution scenarios and Food Loss reduction. Shelf life international meeting. Special 7th edition. 21st-23rd Ottobre 2015. Monza



- 6 **Adobati A., Limbo S., Teruzzi M., Uboldi E, Piergiovanni L.** 2015. Shelf life assessment of strawberries (*Fragaria x Ananassa* Duch.) stored in masterbag with active devices by response surface methodology. Shelf life international meeting. Special 7th edition. 21-23 Ottobre 2015.



- 7 Limbo S., Adobati A. Uboldi E. Piergiovanni. 2015. Packaging attivo in “master bag” ed Estensione della Shelf life di lamponi (*Rubus idaeus* L.): una strategia affidabile per ridurre a perdita di cibo. Agorà - L'incontro nazionale sul food packaging. 21-23 Ottobre 2015. Monza

25AP Packaging attivo in “master bag” ed Estensione della Shelf Life di lamponi rossi (*Rubus idaeus* L.): una strategia affidabile per ridurre la perdita di cibo

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I piccoli frutti sono molto apprezzati dai consumatori per la loro alta qualità sensoriale e il loro valore nutrizionale. I lamponi sono prodotti molto deperibili, avendo alti tassi di respirazione e una struttura fragile. La perdita d'acqua, la formazione di muffe e la produzione di odori e sapori anomali durante la conservazione limitano la durata di conservazione di questi frutti a pochi giorni. Attualmente, le caratteristiche deperibili di questi frutti contribuiscono ad una elevata perdita di questo alimento lungo la catena di approvvigionamento e di distribuzione. In questo lavoro, è stata studiata la possibilità di estendere la shelf life di lamponi rossi utilizzando soluzioni di packaging attivo dopo aver definito in modo oggettivo i criteri di cut-off utili nell'indicare il momento in cui si ha un significativo decremento della qualità. L'obiettivo finale era quello di stimare il ruolo di una nuova tecnologia di packaging nel ridurre l'impatto ambientale lungo la catena di approvvigionamento, ma tenendo conto dei benefici ottenuti dalla riduzione delle perdite alimentari derivanti dalla reale estensione di shelf life. In questo lavoro sono stati utilizzati lamponi rossi (*Rubus idaeus* L.) cv. Erika. I frutti sono stati raccolti manualmente in fase di maturazione e confezionati entro 24 ore, per la sperimentazione sono stati selezionati solo i frutti di bosco con colore comparabile e assenza di difetti e muffe. Le soluzioni di packaging considerate sono le seguenti: A) vassoi in PET macroperforati contenenti 125g di lamponi; B) master bag di materiali plastici aventi differenti valori di OTR e WVTR e contenenti due vassoi in PET macroperforati con 125g di lamponi; C) master bag contenenti due vassoi in PET macroperforati con 125g di lamponi, un assorbitore di ossigeno ed un diverso numero di emettitori di CO₂. Durante la conservazione a 5°C e al 75% di umidità relativa sono state condotte le seguenti analisi: valutazione delle bacche danneggiate ed ammuffite (%); perdita di peso (%); colore (CIE L*a*b*); solidi totali (g/100g); solidi solubili (g/100g); pH; acidità titolabile (g di acidi citrico/100g); consistenza (espressa come forza per deformazione). Un approccio multivariato basato sull'analisi delle componenti principali ha permesso di verificare una relazione tra l'andamento della PC1 e il tempo di conservazione, pertanto con tale approccio è stato stimato un valore di PC critico utile alla definizione della shelf life dei lamponi nelle diverse soluzioni di confezionamento. Si è pertanto stimato un tempo di shelf life pari a 3±0.5 giorni e 6±0.4 giorni, usando rispettivamente le confezioni tradizionali (A) e i master bag in atmosfera passiva (B), mentre l'impiego della soluzione in master bag con soluzioni attive ha consentito di prolungare la shelf life fino a 10±0.7 giorni. Nello stesso lavoro, gli impatti ambientali di ciascuna delle soluzioni sono stati calcolati mediante la metodologia LCA (Life Cycle Assessment), tenendo in considerazione non solo il contributo del packaging ma anche quello apportato dall'estensione della shelf life ottenuta con ciascuna delle soluzioni considerate. I risultati mostrano che l'imballaggio attivo permette un'estensione della shelf life che consente una riduzione degli impatti ambientali, contribuendo in modo significativo ad una riduzione delle perdite di questo prodotto.

Keywords: lamponi, estensione della shelf life, imballaggio attivo, LCA

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Shelf Life Extension of Raspberry: Passive and Active Modified Atmosphere Inside Master Bag Solutions

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Consumers often appreciate small red fruits for their high sensory quality and nutritional value. Furthermore, raspberries are very perishable presenting high respiration rates and a fragile structure; they are easily spoiled by moulds and consequently have a very short shelf life, about 3-5 days.

The general purpose of this study was to investigate the ability of passive and active modified atmosphere packaging in extending the shelf life of red raspberries (*Rubus idaeus* L., cv. Erika). In the case of passive atmosphere, red raspberries were packed inside macro-perforated polyethylene terephthalate (PET) trays inserted in a master bag, made of plastic materials with different permeability and selectivity to oxygen and carbon dioxide. In the case of active packaging, raspberries were packaged into macro-perforated PET trays; 8 of them placed into a cardboard crate, then inserted into an low density polyethylene (LDPE) master bag before sealing, carbon dioxide emitters were introduced. For each trial, the analyses were performed at different times storing the product in a cold dark chamber (Set 1 °C, 70 NRM). We performed chemical, mechanical, sensory analyses. Consumer rejection, described by means of the Weibull's equation, was correlated to chemical and physical changes in order to find an acceptability limit useful in defining the product shelf life.

The use of active and passive modified atmosphere packaging resulted in a significant increase on the shelf life of raspberries. This will have a great impact on the waste reduction of this product along the distribution chain, increasing its sustainability.

1. Introduction

Raspberries are non-climacteric fruit, highly perishable for being susceptible to mechanical injury during transportation and picking, water loss, moulds and rot growing during storage. Mould growth can limit the shelf life of fruits (Kim and Willis, 1998; Hertog et al., 1999). During raspberry life, physiological decay occurs due to the high respiration rates. For all these reasons, the postharvest life of red raspberries is limited to a few days (3-5 days) and only a small percentage of these fruits can be consumed fresh.

During storage, a possibility to control the decreased quality would rely on packaging technologies able to modify the gas composition inside the package, slowing down the fruit metabolism and microorganisms' growth. In fact, it is well known that O₂ and CO₂ concentrations around 10 % and 10-20 %, respectively, are desirable to preserve fresh raspberries quality (Joles et al., 1994). To obtain this proper gas composition, the modification of the atmosphere can be achieved through passive or active solutions (Brody, 2001). In the first case, the permeability of the packaging material and its selectivity towards gases can be combined with the fruit metabolism to control the equilibrium of oxygen and carbon dioxide in the headspace around the product. In the second case, an active device quickly modifies the gas composition inside the packaging by releasing or absorbing, for example oxygen and carbon dioxide.

Previous works had demonstrated the effectiveness of storage in master bags under controlled atmosphere to extend the shelf life of fresh fruits, but exploiting only the gas permeability of the plastic film and the fruit metabolism to modify the concentration of gases inside the package (Giovaneli et al., 2014; Joles et al., 1994; Van deer Sleen, 2002). This system can be economical and easy to use for retailers and producers, but it

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