

Instituto Politécnico de Santarém

Escola Superior Agrária de Santarém

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# **Evaluation of potential of gamma radiation as a conservation treatment for blackberry fruits**

Thesis submitted for Master degree in the area of Food Engineering

**Marina Oliveira**

Advisors:

**Doutora Paula Pinto  
Doutora Sandra Cabo Verde  
Mestre Gabriela Lima**

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## *Abstract*

Blackberries consumption has been associated with health benefits; however, these fruits are quite perishable. The objective of this study consists in analyse the potentiality of gamma radiation as a decontamination method for blackberry fruits. Fresh blackberries were irradiated in a  $^{60}\text{Co}$  at different doses (0.7 kGy up to 2.6 kGy) and microbiological, physicals, chemicals and sensorial parameters were assessed immediately after irradiation and at after two, and three and seven days of storage time at 4°C, for first and second sampling, respectively. The microbial inactivation efficiency on blackberries ranged between 48% and 99% for gamma radiation doses  $\geq 1$  kGy. Moreover, the applied irradiation doses do not result in a major impact on the physical, chemical and sensory attributes of blackberries. This work shows that treatment with gamma radiation has a high potential to be evaluated as a treatment for conservation of blackberries.

**Key-words:** Blackberries, Food irradiation, Food microbiology, Physical and chemical properties, Phytochemicals

## *Resumo*

O consumo de amoras tem vindo a ser associado a benefícios na saúde, contudo, estas frutas são bastantes perecíveis. O objectivo deste estudo consiste em analisar a potencialidade da radiação gama como um método de descontaminação para amoras. As amoras frescas foram sujeitas a uma fonte de  $^{60}\text{Co}$  a diferentes doses (0.7 kGy a 2.6 kGy), e foram avaliados parâmetros microbiológicos, físicos, químicos e sensoriais. Estas análises foram efectuadas imediatamente após irradiação e após dois e três e sete dias de armazenamento a 4 °C, para a primeira e segunda amostragem, respectivamente. A eficiência de inactivação microbiana em amoras variou entre 48% e 99% para as doses de radiação gama  $\geq 1$  kGy. Por outro lado, as doses aplicadas não resultam num grande impacto sobre os atributos físicos, químicos e sensoriais das amoras. Este trabalho revela que o tratamento com radiação gama apresenta um elevado potencial a ser avaliado como tratamento de conservação de amoras.

**Palavras-chave:** Amoras, Irradiação de alimentos, Microbiologia alimentar, propriedades físico-químicas, Fitoquímicos

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## *Index terminologies*

$^{137}\text{Cs}$  – Césio 137

$^{60}\text{Co}$  – Cobalto 60

**aW** – water activity

**DAD** - diode array detection system

**DMax** – Maximum dose

**DMin** – Minimum dose

**FW** - fresh weight

**GAE** – Gallic acid equivalent

**kGy** – Absorbed dose - quantity of ionizing radiation energy absorbed per unit mass of a specified material (KiloGray)

**kGy/h** – Dose rate – the quantity of radiation absorbed per unit time.

**MEA** – Malt Extract Agar

**t** – Time

**TSA** – Tryptic Soy Agar

**$\alpha$**  – Alfa particles

**$\beta$**  – Beta particles

**$\gamma$**  – Gamma radiation

# *Chapter 1 - Introduction*

## 1.1 *Introductory note*

Vegetables and fruits provide most of the micronutrients of the human diet. They are also important sources of dietary fibre and phytochemicals (Boeing *et al.*, 2012). A large number of studies have been linking berry fruits consumption to a profound and positive effect on human health, due to its high content in polyphenols (Beattie, 2005; Seeram, 2012). As reported by numerous studies, these polyphenols have shown anti-inflammatory, anticancer and neuroprotective properties, thus preventing cardiovascular and neurodegenerative diseases, diabetes and cancer (Seeram, 2008; Paredes-Lopez, 2010; Tavares, 2012).

A wide variety of fungi (mostly moulds) is capable of growing and spoiling various types of berries; considering the fact that these commodities contain high levels of sugar and other nutrients and a water activity, ideal for fungal growth. Post-harvest fruit spoilage results in significant economic losses (Tournas, 2005). Additionally, if the spoiling fungi are toxigenic or pathogenic, they could pose a health risk for the consumer (Tournas, 2001). Blackberries are highly perishable fruits, with an usual shelf life of two to three days at refrigerated temperatures (Joo, 2011), showing great limitations regarding fresh market utilization. Some fruits show sensitivity to low temperatures and they can suffer chilling damages and develop higher susceptibility to microbial spoilage. On the other hand, the use of synthetic fungicides can prevent spoilage but some fungi may develop resistance to commonly used pesticides. Furthermore, there is a trend towards the reduction of chemicals in food, due to their hazardousness to human health (Tournas, 2005). Therefore, it is of the most importance the use of technologies that can improve shelf-life without altering physical, sensorial and nutritional characteristics of food. Recent interests in these technologies are not only to obtain high-quality food with “fresh-like” characteristics, but also to provide food with improved functionalities. In addition to their possible beneficial effects on nutritional and bioactive content many of these novel technologies are more cost-efficient and environment friendly for obtaining premium quality foods which have led to their revival and commercialization (Rawson, 2011).

In response to technological progress in manufacturing there are emerging alternative technologies in food conservation without use of chemicals, such as food irradiation. Irradiation is a nonthermal process that has been reported to inactivate foodborne

pathogens and prolong the shelf-life of fresh fruits and vegetables (Arvanitoyannis, 2010). To provide a general indication of commercial processing costs (e.g. throughput more than 60 000 t/a), for a treatment dose up to 1 kGy the cost would be US \$1-3 per ton. The cost of irradiation may be discounted by the cost of the alternative process which irradiation replaces. An additional offsetting saving may be realized in treatments which prolong shelf-life or reduce waste, thus increasing the volume of saleable product and profits (FAO/IAEA, 1998).

## 1.2 *Characterization of blackberries*

High consumption of fruits and vegetables has since long been associated with a lower risk of non-communicable chronic and inflammatory diseases, such as cardiovascular diseases, diabetes and some types of cancer (World Health Organization, 2003; Boeing, 2012).

Blackberries (*Rubus L.*) are usually grown in Asia, Europe and North and South America. The fact that the blackberries have grown in several regions of the world resulted in the existence of various native species (Nunes, 2008). The blackberry is a shrub species that produces fruit constituted by mini drupes forming aggregates. The fruit has an average weight between to 4 and 7 grams, black colouring and a sweet-sour taste (<http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Amora/SistemaProducaoAmoreiraPreta/>). The maturity index most used in blackberries is the change of surface colour. The immature fruit are red and turn black shiny when ripen, being harvested at this stage. Black shiny is the preferable colour for fresh market (Nunes, 2008). The taste of blackberries does not change significantly after the harvest, therefore should be taken with quality features very close to those of consumption. The main indices of quality for blackberries are appearance, firmness, flavour and nutritional value. Practices carried out in the cultivation, harvesting and marketing are directly related to the quality of the fruit (<http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Amora/SistemaProducaoAmoreiraPreta/>). Blackberries are characterized by a high content of anthocyanins and ellagitannins, as well as some flavonols and flavanols (Seeram, 2008). Blackberry phenolic composition and concentrations are known to be influenced by genetics, growing conditions, and maturation (Kaume, 2012). Soluble solids and total sugar contents, pH, and volatile production increase from the immature fruit to the ripen stages, and the acidity decreases sharply in same process (Nunes, 2008). This fruits contain high levels of sugar

and other nutrients and a water activity ideal for fungal growth. Based on that, blackberries are highly perishable fruits that show large limitations in fresh fruit market (Joo, 2011).

### 1.3 *Aims*

With the objective of responding to the consumer's requirements of Food Safety and Quality, and considering the reduced shelf life of blackberry, this study aims to analyse the potential of gamma radiation as a decontamination tool. Also the effects of gamma radiation in some physical, chemical and sensory parameters of blackberry fruits were evaluated since these factors are of great importance to for the consumer. This study provide a better understanding of the effects of ionizing irradiation as a preserving method for fresh fruits, being part of the scope of an International Atomic Energy Agency (IAEA) Research Cooperation Project on the Development of irradiated fresh fruits and vegetables for immunocompromised patients. The specific objectives of the study were:

- i. Evaluation of the effects of different gamma radiation doses on blackberries fruits microbiota;
- ii. Phenotyping of blackberry fruits microbiota before and after irradiation;
- iii. Follow the evolution of maturation of blackberries through total soluble solids, colour and texture before and after irradiation;
- iv. Analyse the phytochemical stability and phenolic compounds present in the samples;
- v. Evaluate the opinion of potential consumers.

The work presented in this thesis was developed in three different entities. Acquisition of the blackberries and the chemical analyses (total polyphenols and phytochemicals profile) were performed in the “Instituto de Tecnologia Quimica e Biológica” (ITQB). The irradiation of the samples and the microbiological analyses were carried out in “*Campus Tecnológico e Nuclear*” (CTN). The physical analysis (colour, °Brix and texture) were conducted in “*Escola Superior Agrária de Santarém*” (ESAS). The sensory evaluation of blackberries was divided into two groups, the first in CTN and the second in ITQB.

This thesis is divided into eight chapters. In Chapter 1 the theoretical background and product characterization is presented. In Chapter 2 is exposed the irradiation process and dosimetry of the samples and their methodologies and results. The microbiological analysis, including the methodologies employed, presentation and discussion of results are in Chapter 3. Chapters 4 and 5 represent respectively a brief introduction to the physical and chemical analyses carried out in this work, and the corresponding methods, results and discussion. The performed sensory analysis for the food product is present in Chapter 6. The conclusions from the performed analysis as well as the work to develop in the future are presented in Chapter 7. Finally, the references used in the preparation of this work can be found in Chapter 8.

# *Chapter 2 - Blackberry*

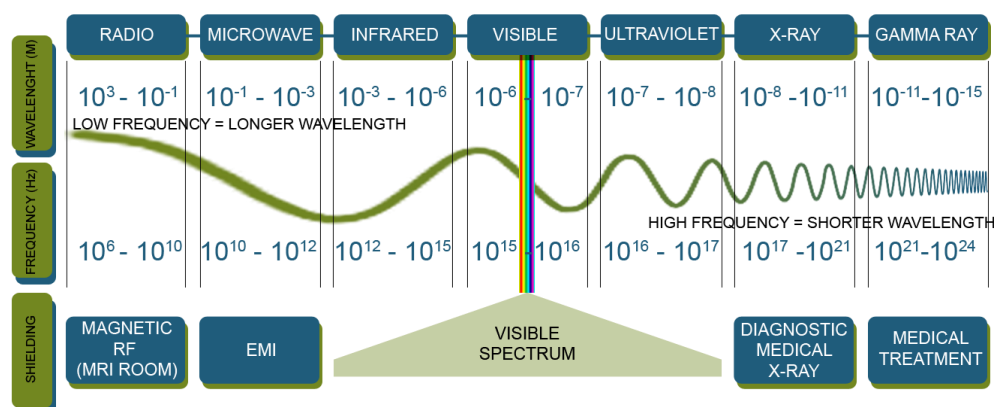
## *Irradiation*

## 2.1 Principles and applications of food irradiation

### 2.1.1 Ionizing radiation

The radiation energy is transmitted in the form of electromagnetic waves or particles that can be produced from natural sources or devices built by human (<http://orise.orau.gov/reacts/guide/define.htm>).

The Figure 1 represents the electromagnetic spectrum where it can be seen the associated wavelength, frequency and some application from radio waves, with low frequency, up to X-rays and gamma rays that have very high frequencies and wavelengths very short. Between these limits are the microwaves, infrared, visible and ultraviolet light.

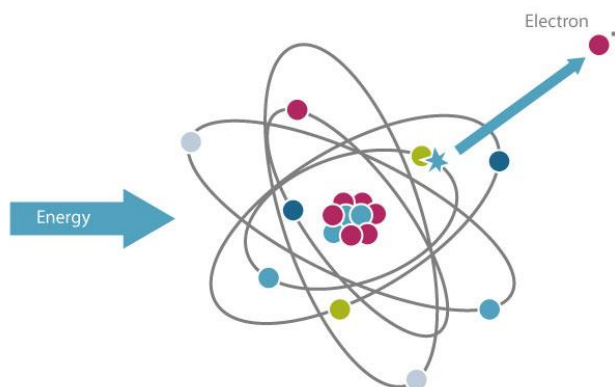


**Figure 1.** Electromagnetic spectrum with applications and their wavelengths and frequencies (adapted from <http://knowledgeserver.wordpress.com/2012/07/31/electromagnetic-spectrum/>)

Depending on the amount of energy, radiation may be described as ionizing or non-ionizing. The latter have low energy and are common forms of radiation linked to our everyday life (e.g. visible light, microwaves and radio waves).

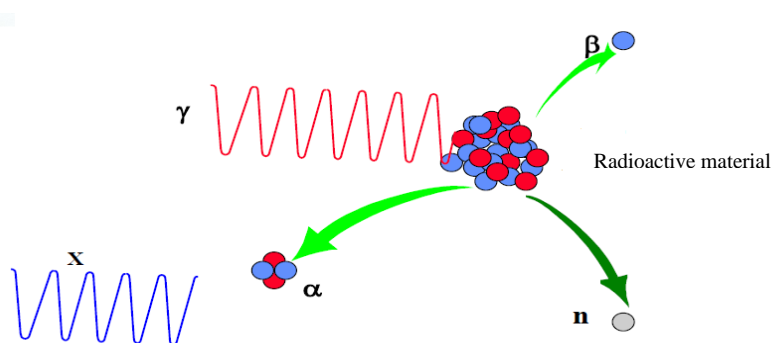
Ionizing radiations have high energy levels and may change the physical state of an atom and cause the loss of electrons, making them electrically charged, referring to this process

as "ionization" (Figure 2) ([http://www.fiocruz.br/biosseguranca/Bis/lab\\_virtual/radiacao.html](http://www.fiocruz.br/biosseguranca/Bis/lab_virtual/radiacao.html)).



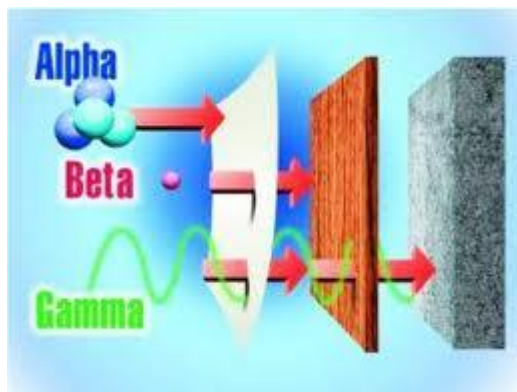
**Figure 2.** Ionization scheme for ionizing radiation (adapted from <http://www.medicalradiation.com/facts-about-radiation/what-is-radiation/ionizing-radiation/>)

Invisible, odourless, undetectable, insipid and painless are some of the characteristics of ionizing radiation (<http://www.segurancaetrabalho.com.br/download/rad-ioniz-cuidados.pdf>). The emission energy may occur in the form of alpha particles ( $\alpha$ ), beta ( $\beta$ ), X-rays, gamma rays ( $\gamma$ ) and neutrons (Figure 3).



**Figure 3.** Types of ionizing irradiation (adapted from Trindade 2011)

In Figure 4 it can be seen the penetration power of different ionizing radiation.



**Figure 4.** Penetrating power of different ionizing radiations (adapted from <http://www.dbcp.gov.hk/eng/safety/knowledge.htm>)

Beyond the penetration power, these radiations have differing characteristics both in relation to the constitution, nature or origin, or their ability to cause greater or lesser ionization (Martins, 2005).

There are three types of allowed radiation for food processing by Codex General Standard for Irradiated Foods: gamma rays, X-rays and electrons (Codex Alimentarius Commission, 2003). These types of radiation are also used in commercial radiation to process products like medical and pharmaceutical products (International Atomic Energy Agency – IAEA in Arvanitoyannis, 2010).

Gamma rays are the most used of the three radiations for a variety of industrial processes because it can penetrate more deeply than e-beams (3.80 cm) and is less expensive than X-rays (Arvanitoyannis, 2010).

The commercial use of gamma radiation to sterilize health care products began in the late 1950s. With increasing experience and confidence on technology, there was an expansion for new applications such as irradiation of food and radiation treatment of cancer.

Gamma radiation is comprised of electromagnetic waves emitted by unstable nuclei. When ionizing rays interacts on the product, free electrons can take part in chemical reactions or destroy DNA molecules from living organisms (Arvanitoyannis, 2010).

More than 200 gamma-ray installations are being used for various industrial applications, mostly for sterilizing medical devices and for food irradiation (Christopher, 2006). Cobalt-

$^{60}\text{Co}$  and cesium-137 are the gamma rays sources approved by international standards for food irradiation (CFR 1986; Codex 2003). At the moment, cobalt-60 is the most chosen isotope for food irradiation because of its highly developed production, fabrication and encapsulation technology (Arvanitoyannis, 2010). Cobalt-60 is produced in a nuclear reactor by neutron bombardment of highly refined cobalt-59. This has a tendency to disintegrate emitting beta particles and gamma radiation (Molins, 2001).  $^{60}\text{Co}$  has a half-life of 5.26 years, so the activity decays by 12.35% per year (Christopher, 2006).

Examples of areas in the application of ionizing radiation are (<http://www.chipionizing.pt>):

- i. Conservation and Restoration;
- ii. Pharmaceuticals;
- iii. Research and Development;
- iv. Cork, and
- v. Food.

## 2.1.2 Applications

Food irradiation is the exposure of food to ionizing radiation and is used for more than 60 food products being legally accepted in more than 40 countries. Food irradiation is a rising technology due to its potential for the inactivation of microorganisms. This technique has been an effective way of inactivation of pathogenic and non-pathogenic bacteria, fungi, parasites and certain viruses (Arvanitoyannis, 2010).

The inactivation of microorganisms occurs by radiation damage to the cell's genetic material. A photon or an electron reaches the genetic material of the cell causing damage to the DNA (Grecz et al. 1983). The available evidence is unanimous that this process can control many biological hazards relating to food without adverse effects (Molins, 2001). "Irradiation is commended as a safe and effective food processing method that can reduce the risk of food poisoning and preserve foods without detriment to health and with minimum effect on nutritional quality" (Kouba, 2003 – in Christopher, 2006).

Irradiation is a "cold process" that, unlike thermic treatment can be used for pasteurization and sterilization of foods without causing modifications (Pinto, 2007). This treatment leave no toxic residues that are harmful to food, showing more effectiveness in the reduction of the number of pathological microorganisms and/or deterioration and retards and/or

eliminates natural biological processes, such as maturation, germination or microbial growth in fresh food (World Health Organization, 1994; Arvanitoyannis, 2010; Arvanitoyannis, 2009). Some criteria are relevant to a safe and effective irradiation:

- (i) The absence of induced radioactivity;
- (ii) The absence of viable pathogens or their toxins;
- (iii) The absence of excessive loss of nutrients;
- (iv) The absence of toxic, mutagenic, or carcinogenic radiolytic products.

Irradiation in food can be used for various purposes (advisable limits of doses for good practice of irradiation):

- (i) Reduce insect infestation of grain (1.0 kGy);
- (ii) Inhibit sprouting in tubers and bulbs (1.0 kGy);
- (iii) Retard postharvest ripening of fruits (1.0 kGy);
- (iv) Inactivate parasites in meats and fish (2.0 kGy);
- (v) Eliminate spoilage microbes from fresh fruits and vegetables (2.5 kGy);
- (vi) Extended the shelf-life in fresh fruits and vegetables (2.5 kGy);
- (vii) Extended the self-life of poultry, meats, fish and shellfish (3.0 kGy);
- (viii) Reducing microbial load of grain (5.0 kGy);
- (ix) Reduction pathogens in fresh or frozen fish (5.0 kGy)
- (x) Reduction pathogens in fresh or frozen poultry and beef (7.0 kGy);
- (xi) Reduction pathogens in dried spices (10 kGy); and
- (xii) Sterilize foods and feeds (10 kGy) (Molins, 2001).

At the permitted doses, food irradiation can reduce common foodborne pathogens as *Campylobacter jejuni*, *E.Coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* (various species). However some microorganisms show resistance to irradiation like bacterial spores, some moulds and yeast, virus and prions (Arvanitoyannis, 2010).

The irradiation process can be applied in the final packaging, avoiding the possibility of recontamination, but, is not a substitute for a good manufacturing process.

The food packaging materials for irradiation need to be well-selected according to the applied dose. Some materials lead to the formation of free radicals and ions. The effect of ionizing radiation on food packaging materials has been extensively studied over the years and is crucial for packaging engineering (Arvanitoyannis, 2010).

Irradiation cannot improve poor-quality food products because it does not reverse the physiological and chemical processes involved in decay. Like any other food processes, irradiation induces some chemical changes in food (*e.g.* some vitamins A, B1, C and E), in some cases less than other preservation methods (Arvanitoyannis, 2010).

The Scientific Committee on Food (SCF) based on a large number of feeding studies reported that the food irradiation is a safe process. WHO/FAO/IAEA arrived to the same conclusion stating that irradiated food is safe to eat (Arvanitoyannis, 2010).

### 2.1.3 Regulation

The Codex Alimentarius Commission FAO/WHO, and many other regulatory bodies, have established fundamental principles for the foods irradiation, as well as basic procedures of control.

In the European Union, Directive 1999/2/EC covers legislative issues on food and food ingredients treated with ionizing radiation. The Directive provides a series of specifications for ionizing radiation that can be used in the control of allowed radiation levels and the requirements for labelling. The conditions for importation of irradiated foods are also specified (<http://www.eufic.org/article/pt/artid/irradiacao-dos-alimentos/>).

In 2011 the European Community had 13 members`states with authorization for irradiation of food. From these 13 states, there were 24 approved irradiation facilities. A total of 8067.5 tons of product were irradiated. Dosimetry analysis on 5397 samples showed that 97% the samples of the European community and 100% of samples analysed in Portugal were in accordance with the applied dose (Comissão Europeia, 2012).

To date, only one category of food - dried aromatic herbs, spices and vegetable extracts - are included in the list of foods that may be irradiated in Portugal.

Each Member State has notified national authorizations for certain foods in accordance with Directive 1999/2/EC. The list of national authorizations is issued by the Commission.

Under Article 6 of Directive 1999/2/EC, any irradiated food or any irradiated ingredient of a food compound must be labelled with the words 'irradiated' or the words 'treated with ionizing radiation' or radura symbol (Figure 5) (Comissão Europeia, 2007).



**Figure 5.** International symbol, Radura, to indicate that the product was irradiated (adapted from <http://s704.photobucket.com/user/godworksaura/media/radura.gif.html>)

## 2.1.4 Dosimetry

According to the Decree-Law n° 337/2001 of December 26<sup>th</sup>, the maximum allowed radiation dose for foodstuffs is 10 kGy.

The radiation process is monitored by measuring the amount of absorbed energy. The amount of absorbed energy is calculated by the interaction of the radiation with a known detection system for dosimeter. This dosimeter indicates the total radiation to which a person/object is subjected. The effects produced by the interaction of the radiation with the detector allow conclusions about the amount and properties of radiation detected (Daros, 2006).

Agreement to regulations of a commercial facility is verified by use of the appropriate dosimeter as well as with national and international standards, and during processing or research.

In radiation research, dosimetry aims to determine the dose to achieve a desired effect, and the inappropriate dosimetry has as consequence incorrect conclusions. In the case of radiation processing, that consequences can be both economic and legal.

The International Organization for Standardization (ISO), European Committee for Standardization (CEN), and American Society for Testing and Materials, (ASTM) are the international organizations responsible that set recognized dosimetry procedures (Christopher, 2006).

The absorbed dose (D) is the amount of absorbed energy per unit mass after exposure to radiation, measured in Joules per kilogram [ $\text{Jkg}^{-1} = \text{gray (Gy)}$ ]. This unit represents the amount of energy that one kilogram of matter receives of ionizing radiation (Trindade, 2011).

The correct measurement of the dose distribution strongly contributes to the maximum absorbed dose (Dmax) and minimum absorbed dose (Dmin) is properly applied to the food product (Christopher, 2006). The Decree-Law nº 337/2001 of December 26, reports that for homogenous products or in bulk homogeneous density, it is possible to determine the overall average dose absorbed by an adequate amount of dosimeters distributed strategically and at random throughout the products.

There are several types of dosimeters such as chemical (*e.g.* Fricke dosimeter) and physical (*e.g.* ionization chamber).

The dosimeters frequently used in routine processes consist of a polymer termed polymethylmethacrylate (PMMA). Red Perspex 4034, Amber Perspex 3042 and GammaChrome YR (Figure 6) are some examples of these dosimeters (Silva, 2006). For hydrogenous material (*e.g.* fruit, meat, poultry or vegetable), these routine dosimeters are useful (Christopher, 2006).



**Figure 6.** Gammachrome YR, Amber and Red Perspex (adapted from Harwell Dosimeters).

Dosimeters are irradiated within the bags of aluminium, properly identified so as to avoid systematic errors caused by environmental effects such as temperature, relative humidity, presence of oxygen and other gases. Table 1 presents the main features of the different dosimeters.

**Table 1.** Characteristics of different routine dosimeters, adapted of Harwell Dosimeters.

	Gammachrome YR	Amber Perspex 3042	Red Perspex 4034
<b>Dose range (kGy)</b>	0,1 – 3,0	1,0 – 30	5,0 – 50
<b>Reading wavelength (nm)</b>	530	603 (1 - 10 kGy) 651 (10 - 30 kGy)	640
<b>Shelf life</b>	-	5 years	10 years
<b>Measurement reproducibility</b>	± 3%	± 2,5%	± 2%

Dosimeters should be well selected according to the dose intended to use. After dosimeter irradiation the absorbance is measured at a specific wavelength to determine the absorbed dose by the sample (the absorbed dose is proportional to the absorbance measurement).

## 2.2 Irradiation methodology

### 2.2.1 Sampling

The first sampling of blackberries (*Rubus fruticosus*, cv. Primark) consisted of two batches (batch 1 and 2, both of 1500 g) that were grown in Fataca experimental field (Odemira, Portugal), and transported to the ITQB and thereafter to the CTN. Fully ripened fruits were harvested and packed in polystyrene boxes (125 g/package) with a lid and holes for air circulation.

The second sampling was also comprised of two batches (batch 3 and 4, both 5000 g), where the first came from Netherlands and the second from Mexico. The species of blackberries were not identified, but it is known that blackberries are internationally traded, including Portugal. Both batches of blackberries were harvested and packed in polystyrene boxes (125 g/package) with a lid and holes for air circulation.

Both samples were transported to the microbiology laboratory of CTN, and kept at refrigeration temperatures until analysis. After irradiation and microbiological analyses performed at CTN, the samples were divided and transported to ITQB and ESAS to carry out the chemical and physical analyses.

Table 2 summarizes the samples collected and the tests performed.

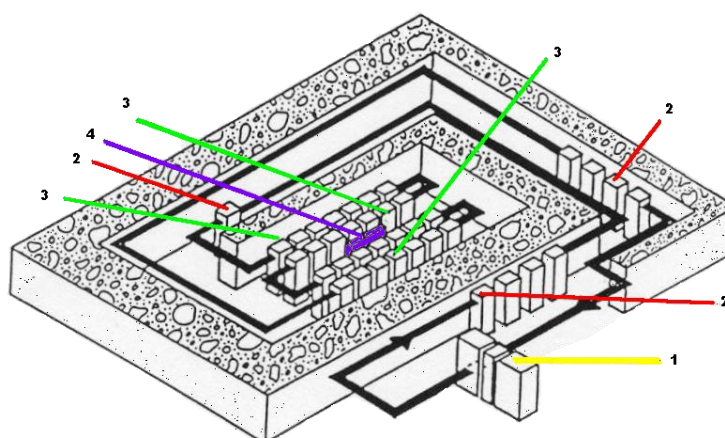
**Table 2.** Analysis performed for each blackberry batch

	<i>Sampling 1</i>				<i>Sampling 2</i>					
	Batch 1		Batch 2		Batch 3			Batch 4		
Evaluated days (after irradiation)	0	7	0	2	0	3	7	0	3	7
Code	1T0	1T7	2T0	2T2	3T0	3T3	3T7	4T0	4T3	4T7
Origin	Portugal		Portugal		Netherland			Mexico		
Amount	1500g		1500g		5000g			5000g		
<b>Analysis</b>										

Code	1T0	1T7	2T0	2T2	3T0	3T3	3T7	4T0	4T3	4T7
Microbiology	X	X	X	X	X	X	X	X	X	X
TSS	X	X	X	X	X	X	X	X	X	X
Colour	X	X	X	X	X	X	X	X	X	X
Texture	X	-	X	X	X	X	X	X	X	X
Total phenolic	-	-	-	-	X	X	X	X	X	X
Phytochemical	-	-	-	-	X	X	X	X	X	X
Sensory	-	-	X	X	X	X	X	X	X	X
Analysis										

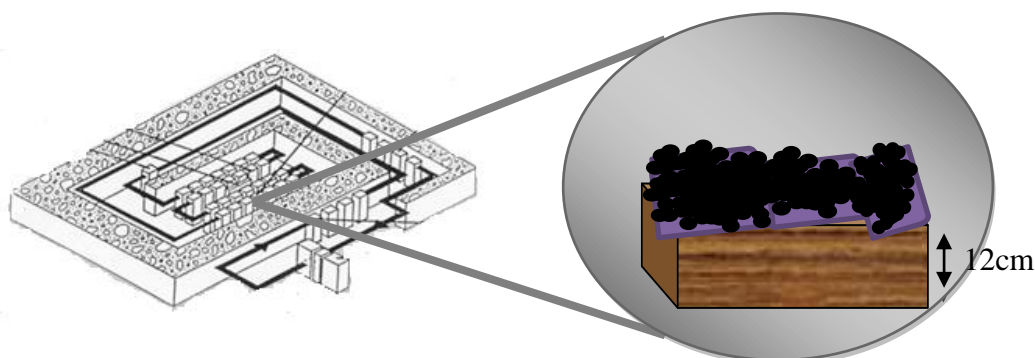
## 2.2.2 Irradiation of Blackberries

The irradiation of the samples was performed at the Unidade Tecnológica de Radiação (UTR) located on the *campus* of CTN. The irradiation chamber (Figure 7) shows a total area of 30.8 m<sup>2</sup> and is delimited by concrete walls with a high density (2.23 g.cm<sup>-3</sup>), which is part of a labyrinth which acts as a shield against radiation. Inside the irradiation chamber there is a planar irradiator formed by thirty stainless steel tubes with 16 mm diameter each, inside which is the source of energy - the radioisotope <sup>60</sup>Co. The radiator has a nominal activity exceeding 97 kCi (3.589e<sup>15</sup> Bq) in December of 2013.



**Figure 7.** Scheme of the irradiation chamber and labyrinth of UTR. Legend: 1) System electropneumatic load / rearrangement / discharge; 2) Waiting positions, 3) Irradiation position and 4) irradiator (adapted from MATOS, 2003)

Samples were placed in a position in front of the irradiator (12 cm of soil) at a dose rate of 2.5 kGy/h (Figure 8).



**Figure 8.** Scheme of the irradiation geometry of the samples in the ionization chamber

For the first and second batches, the boxes with blackberries were irradiated at three theoretical doses (0.5, 1.0 and 1.5 kGy). For each batch of irradiation were used three boxes of 125 grams per dose and three boxes 125 grams were not subjected to irradiation (non-irradiated samples). Absorbed doses were monitored by four routine dosimeters (Amber Perspex Harwell®, Batch V).

The irradiations of the third and fourth batches were carried out at the doses of 1.0, 1.5 and 2.0 kGy. For each dose there were ten boxes of 125 g of blackberries, and ten boxes of 125 grams of fruits were not subjected to irradiation (non-irradiated samples). Absorbed doses were monitored with five and six routine dosimeters, for third and fourth batch, respectively (Amber Perspex Harwell®, Batch W).

The exposition time to achieve an intended dose was estimated based on the dose rate, previously determined by a reference dosimetric system (*e.g.* Frick dosimeter).

After irradiation, the irradiated and non-irradiated samples were stored at 4 °C (storage temperature indicated before and after marketing) until analysis.

### 2.2.3 Dosimetry of blackberries

The absorbed dose was determined based on the values of optical density at 603 nm, in a Shimadzu spectrophotometer, measured for the dosimeter after irradiation and thickness values determined by a thickness gauge brand Mitotuyo using the Equation 1.

$$AbsorbedDose = A + B_1 \times \left( \frac{opticalAbs}{Thickness} \right) + B_2 \times \left( \frac{opticalAbs}{Thickness} \right)^2 + B_3 \times \left( \frac{opticalAbs}{Thickness} \right)^3 + B_4 \times \left( \frac{opticalAbs}{Thickness} \right)^4$$

**Equation 1.** Polynomial function for absorbed dose determination for Perspex dosimeters

The coefficients values for the calibrated dosimeters batch are:

Batch V: A = 0.221, B1 = 2.07, B2 = 0.132, B3 = -0.0108, B4 = 0.00241

Batch W: A = 1.14124, B1 = 2.30173, B2 = 0.03645, B3 = 0.00442, B4 = 0.0021

These coefficients are specific for each dosimeter batch and must be determined by an *in-house* calibration (inside de radiation facility at a specific calibrated location) against a reference dosimeter (*e.g.* Fricke or ceric-cerous sulphate dosimeters).

## 2.3 Dosimetry results

Table 3 to Table 6 show the absorbed dose values for the four irradiated batches.

**Table 3.** Absorbed dose for batch 1 (Average Dose uniformity Dmax/Dmin - 1.21)

<b>Batch 1</b>	<b>Optical Absorption (nm)</b>	<b>Thickness (cm)</b>	<b>Dose(kGy)</b>	<b>Dose<sub>mean</sub> (kGy) ± SD</b>
<b>0.5 kGy</b>	0.0790	0.375	0.66	0.72 ± 0.08
	0.0820	0.375	0.68	
	0.0930	0.322	0.83	
	0.0760	0.322	0.72	
<b>1.0 kGy</b>	0.1410	0.310	1.19	1.10 ± 0.15
	0.1490	0.310	1.25	
	0.1090	0.299	0.99	
	0.1030	0.299	0.95	
<b>1.5 kGy</b>	0.2060	0.329	1.57	1.60 ± 0.03
	0.2110	0.329	1.60	
	0.2010	0.304	1.64	
	0.1920	0.304	1.58	

**Table 4.** Absorbed dose for batch 2 (Average Dose uniformity Dmax/Dmin - 1.07)

<b>Batch 2</b>	<b>Optical Absorption (nm)</b>	<b>Thickness (cm)</b>	<b>Dose (kGy)</b>	<b>Dose<sub>mean</sub> (kGy) ± SD</b>
<b>0.5 kGy</b>	0.0810	0.336	0.73	0.75 ± 0.02
	0.0820	0.336	0.73	
	0.0780	0.300	0.77	
	0.0780	0.300	0.77	
<b>1.0 kGy</b>	0.1230	0.319	1.04	1.02 ± 0.02
	0.1180	0.319	1.00	
	0.1008	0.271	1.01	
	0.1030	0.271	1.03	

<b>1.5 kGy</b>	0.1820	0.298	1.53	1.55 ± 0.07
	0.1980	0.298	1.65	
	0.1820	0.294	1.55	
	0.1710	0.294	1.47	

**Table 5.** Absorbed dose for batch 3 (Average Dose uniformity  $D_{max}/D_{min}$  - 1.43)

<b>Batch 3</b>	<b>Optical Absorption (nm)</b>	<b>Thickness (cm)</b>	<b>Dose (kGy)</b>	<b>Dose<sub>mean</sub> (kGy)</b>
<b>1.0 kGy</b>	0.1030	0.304	0.93	0.77 ± 0.12
	0.0680	0.301	0.66	
	0.0670	0.311	0.64	
	0.0840	0.284	0.83	
	0.0820	0.297	0.78	
<b>1.5 kGy</b>	0.1460	0.279	1.36	1.13 ± 0.19
	0.1510	0.318	1.24	
	0.1030	0.325	0.88	
	0.1340	0.309	1.15	
	0.1100	0.284	1.04	
<b>2.0 kGy</b>	0.1400	0.292	1.25	1.23 ± 0.13
	0.1450	0.279	1.35	
	0.1400	0.312	1.18	
	0.1530	0.296	1.34	
	0.1150	0.297	1.04	

**Table 6.** Absorbed dose for batch 4 (Average Dose uniformity  $D_{max}/D_{min} - 1.57$ )

<b>Batch 4</b>	<b>Absorção Óptica</b>	<b>Espessura (cm)</b>	<b>Dose (kGy)</b>	<b>Dose<sub>mean</sub> (kGy) ± SD</b>
<b>1.0 kGy</b>	0.126	0.300	1.09	1.10 ± 0.18
	0.131	0.3055	1.13	
	0.127	0.312	1.08	
	0.11	0.297	1.01	
	0.19	0.32	1.49	
	0.159	0.3265	1.26	
<b>1.5 kGy</b>	0.219	0.330	1.65	1.71 ± 0.22
	0.199	0.299	1.65	
	0.213	0.330	1.61	
	0.214	0.314	1.69	
	0.258	0.307	2.05	
	0.288	0.334	2.10	
<b>2.0 kGy</b>	0.36	0.296	2.92	2.64 ± 0.36
	0.379	0.316	2.88	
	0.34	0.304	2.69	
	0.345	0.307	2.70	
	0.435	0.306	3.41	
	0.429	0.292	3.52	

Figure 9 is an illustrative of samples blackberries after irradiation.



**Figure 9.** Blackberries from Portugal after 2 days of irradiation (dose increase from left to right - 0, 0.74 kGy, 1.06 kGy, 1.58 kGy)

# *Chapter 3 - Blackberry*

## *Microbiota*

### 3.1 *Main microorganism present in food*

Food microbiology is largely associated with the production and processing of food, using the multiplication of microbial cultures (fungi, yeasts and bacteria). However, this microbial growth can also establish a source of food contamination (Lidon and Silvestre, 2007).

The microorganisms present in foods can be classified as:

- i. Agents of food spoilage - microbiologically spoiled food damaged by microorganisms which is rejected by the consumer (*e.g.* putrefaction, fermentation and rancidity);
- ii. Foodborne pathogens - food infection: can be produced by the ingestion of microorganisms - food poisoning: as a result of ingestion of toxins produced by microorganisms in food;
- iii. Agents for food producers - well controlled microbial growth contributes to the production or processing of different food and may increase pleasant to the taste, good nutritional value, more digestive and easier maintenance than source products (*e.g.* bread, cheese, yogurt, coffee, chocolate, beer, wine). Biotechnology also allows taking advantage of the metabolism of microorganisms by producing all kinds of special compounds which serve as a additives or food supplements (*e.g.* vitamins, amino acids, enzymes, flavorings) (Lacasse, 1999).

Foods have a very large number of components, being mostly water, protein, fat, carbohydrates, minerals, vitamins and nucleic acids. The fact that a wide variety of species of microorganisms can to take advantage of these components, makes the foods be an ideal places for their growth (<http://www.revista-fi.com/materias/54.pdf>).

Bacteria and fungi are the microorganisms with a more important role in nutrition; however, several other microorganisms may also be involved in food although with a minimal role (Lacasse, 1999).

Favourable conditions increase the speed of multiplication of microorganisms. This growth are influenced by intrinsic factors (inherent characteristics of food – *e.g.* pH,

aW, salt concentration) and extrinsic (relative to environmental factors – e.g. temperature, humidity and the presence of gas) (Altanir, 2009).

In food preparation stages an accurate and controlled hygiene is important for lowering effects of contamination, increasing the shelf-life and the effectiveness of treatments for the conservation and reducing the risk of transmission of pathogens (Lacasse, 1999).

Bacteria are widely distributed in nature, and can be found in all environments (e.g. water, soil, air, plant and animal) (Altanir, 2009). The diversity, the amount and frequency of their action in food, make this type of microorganisms the most important group. The identification and classification of bacteria is important and one of the most important used criteria is the gram staining (constitution of the cell wall). About 90% of the wall composition of Gram-positive bacteria is peptidoglycan, whereas in the Gram-negative it is only 5 – 20% of the cell wall (<http://www.scienceprofonline.com/microbiology/bacterial-cell-wall-structure-gram-positive-negative.html>). The latter, despite its thin wall, have a more complex cell wall organized into several distinct layers (phospholipids, lipoproteins, lipopolysaccharides, peptidoglycans). The presence of lipids in gram-negative bacteria is higher (11 to 22%) than in gram-positive bacteria (less than 3%). These different chemical characteristics affect not only gram staining but also numerous physiological functions (Lacasse, 1999).

Initially, bacteriologists based the classification of bacteria on morphological criteria (e.g. shape, colour, spores) and physiological (e.g. enzyme secretion, metabolism) and divide them into classes, order, family, genus and species. However, due to the difficulty in choosing criteria that allowed determining the degree of evolutionary relatedness among groups, it was decided to differentiate groups of bacteria using recognition criteria easily accepted. The *Bergey's Manual* is one of the most recognized manuals in the community of microbiologists. The most important criteria for establishing the current classification of bacteria are:

- i. The cell wall type (gram positive, gram negative)
- ii. The shape of bacteria (cocci, bacilli, spirillae)
- iii. The metabolism (photosynthesis, chemosynthesis, respiration, fermentation)
- iv. Behaviour in relation to oxygen (aerobic, anaerobic)
- v. Ability to produce spores (Lacasse, 1999).

Fungi can be unicellular (yeasts) and multicellular (filamentous fungi). Some yeasts and moulds are necessary for the production of various foods such as bread, beer, wine, and numerous cheeses. They can also be used industrially for production of enzymes, acids and other organic additives useful for the preparation or preservation of foods, as well as to convert organic detritus in food supplements for animals or humans. They are a good source of vitamins and protein (Lacasse, 1999), but they are a potent food spoilage too, responsible for large economic losses of some food products (<http://www.thermoscientific.com/content/dam/tfs/SDG/MBD/MBD%20Documents/Catalogs%20&20Brochures/Microbiology/Food/International-Food-Hygiene-Vol24-No4-Yeast-Moulds.pdf>; <http://www.countrysideinfo.co.uk/fungi/importce.htm>). Fungi are usually less demanding with ideal growing conditions than bacteria and yeasts, but multiply more slowly. They grow easily in fruits and vegetables (Altanir, 2009).

Around 60's has been found that there are some strains that can produce mycotoxins, particular in cereals and products derived from oil seeds (Lacasse, 1999). The conditions for growth of such micro-organisms pass through an optimum temperature between 20 to 30 °C, a humid environment and an optimum pH ranging from 3 to 8. A large number of fungi can thrive at refrigeration temperatures and/or low water availability (Altanir, 2009).

Fungi may have varied colours and aspects (*e.g.* fuzzy, velvety, compact, sandy and gelatinous). However, microscopic observation is required to identify the genus and species of the moulds on the following criteria:

- i. Appearance of the hyphae (with or without septa);
- ii. Features of grower handset, type and appearance of spores;
- iii. Presence of special structures (flagellum, rhizoids) (Lacasse, 1999).

With the increase of consumption of fresh fruits and vegetables in industrialized countries the chances of outbreaks of food poisoning and food infections related to consumption of fresh fruits and uncooked vegetable salads has increased.

The skin of these foods harbours a variety of microbes and the normal microbiota of fruits is varied, and includes both bacteria and fungi. These microbes can appear through a variety of sources (*e.g.* blowing air, composted soil, insects or the fruit fly inoculate the skin/outer surface with a variety of Gram negative bacteria). The contact

with soil, especially partially processed compost or manure, adds diverse human pathogenic microbes generally of the fecal-oral type (e.g. *Enterobacter*, *Shigella*, *Salmonella*, *E. coli* 0157:H7, *Bacillus cereus*). Fungal microbiota of fruits including moulds (e.g. *Rhizopus*, *Aspergillus*, *Penicillium*, *Eurotium*, *Wallemia*) and yeasts (e.g. *Saccharomyces*, *Zygosaccharomyces*, *Hanseniaspora*, *Candida*, *Debaryomyces*, and *Pichia* sp.) are predominant. These microbes are restrained to remain outside on fruit surfaces as long as the skins are healthy and intact. Also, the transmission of viruses (e.g. *Hepatitis A* Virus, Rotavirus, and Norwalk) have been documented by consumption of raw fruits (Hui, 2006).

The saprophytic fungi, yeast, and bacteria are the microorganisms that most contribute to spoilage of fresh berries (Figure 10) (Zhao, 2007).



**Figure 10.** Blackberries from Mexico after 10 days of refrigerated storage (non treated sample)

## 3.2 *Methodology of microbiological analyses*

Microbiological analyses of the blackberries were based on the characterization of the bioburden of the four different batches samples. The purpose of this methodology was the quantitative and qualitative comparison of the microbial population of blackberries after exposure to different doses of irradiation (gamma radiation) and after storage. Blackberry samples of 25 g were blended on 100 mL of physiological solution with 0.1% of Tween 80 and homogenised 15 minutes in a stomacher equipment (Stomacher 3500; Seaward, UK). Serial decimal dilutions were performed before inoculation into

culture medium using the direct plating technique. The following procedures were applied:

- i. Direct spreading aliquots (0.1 ml and 1 ml) of samples washing solution on solid medium Tryptic Soy Agar (TSA, Merck) – enumeration of bacteria;
- ii. Direct spreading aliquots (0.1 ml and 1 ml) of samples washing solution on solid medium Malt Extract Agar (MEA, Merck) – enumeration of filamentous fungi; (Annex I).

Each assay was done in triplicate for each sample. All procedures for microbiological analyses were carried out under aseptic conditions.

The TSA and MEA inoculated petri dishes were incubated for seven days at 32 ° C and 28 ° C, respectively. Microbiological counts were expressed in log of Colony Forming Units per gram of fresh fruit (CFU/g fresh fruit).

Based on the calculation of Colony Forming Units per gram of sample (CFU/g) microbial inactivation efficiency was estimated for each dose using the following equation (Equation 2):

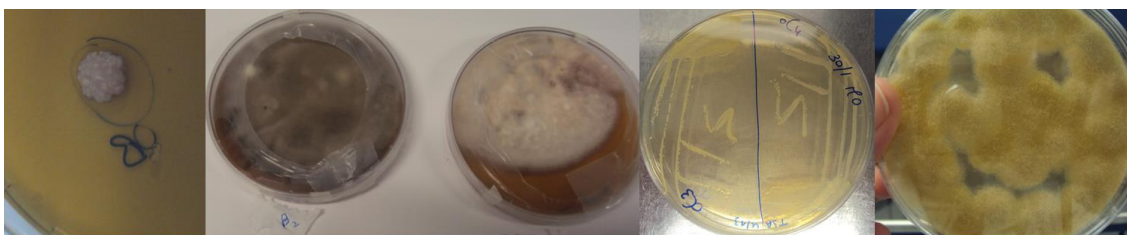
$$\text{Efficiency (\%)} = \frac{(N_0 - N) \times 100}{N_0}$$

**Equation 2.** Estimation of the microbial inactivation efficiency after treatment (dose)

Where:  $N_0$  = number of colony forming units per gram of non-irradiated sample and  $N$  = number of colony forming units per gram of sample after treatment.

Morphological characterization of isolates (Figure 11) from treated (irradiated at various doses) and untreated samples were performed for qualitative assessment of the microbial population. The most frequent colonies (microbial population from non-irradiated and irradiated blackberries) were macroscopically (*e.g.* pigmentation, texture, shape), microscopically and biochemically typed by gram colouring, catalase activity and oxidase test. The isolates were organized into typing groups according to the *Bergey's Manual of Determinative Bacteriology* (Annex II).

All microbiological analyses were performed according to “*Methods for General and Molecular Bacteriology*”.



**Figure 11.** Example of some steps of morphological characterization of microbial isolates from blackberries samples.

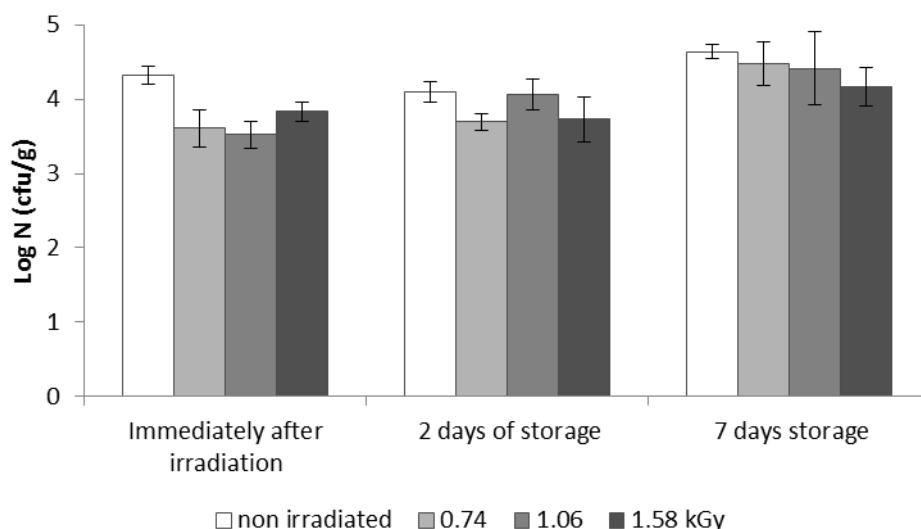
### 3.3 *Microbiological results and discussion*

The microbiological results indicated that blackberries presented initial mesophilic population counts of  $6.9 \times 10^3 \pm 1.6 \times 10^3$  CFU/g for blackberries from Portugal (batch 1 and 2). For second sampling the bioburden was  $1.2 \times 10^3 \pm 1.9 \times 10^2$  and  $6.4 \times 10^3 \pm 1.3 \times 10^3$  CFU/g for blackberries from Netherland (batch 3) and Mexico (batch 4), respectively.

Concerning the fungal population the analysed blackberries from Portugal presented an initial load of  $1.7 \times 10^3 \pm 3.0 \times 10^2$  CFU/g. For second sampling, the fungal population counts were  $1.4 \times 10^4 \pm 5.2 \times 10^3$  CFU/g and  $7.9 \times 10^3 \pm 1.4 \times 10^3$  CFU/g for blackberries from Netherland and Mexico, respectively.

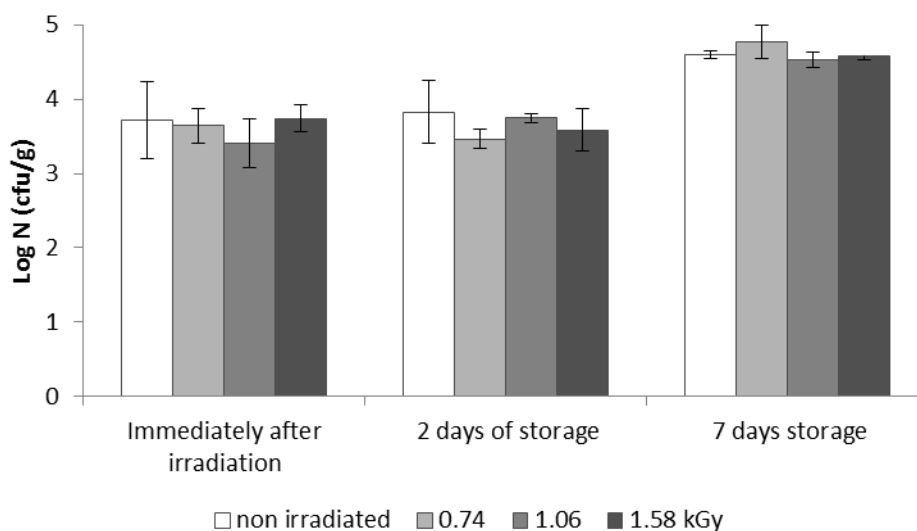
In order to analyse the response of blackberries microbial population to gamma radiation, bars charts were constructed based on the number of microbial survivors in function of the absorbed dose along storage time.

For the Portuguese blackberries (batches 1 and 2) the obtained results do not show an inactivation tendency for the applied gamma radiation doses (Figure 12). However, for blackberries irradiated at 1.06 kGy it was observed an approximately 85% inactivation efficiency (reduction of 0.8 log) immediately after irradiation. After seven days of storage time an increase in the number of microorganisms present in irradiated fruit was observed. On the other hand, for the maximum applied dose of 1.58 kGy the inactivation efficiencies obtained were very reduced, ranging from 48% up to 62% for blackberries immediately after irradiation and after two and seven days of storage.



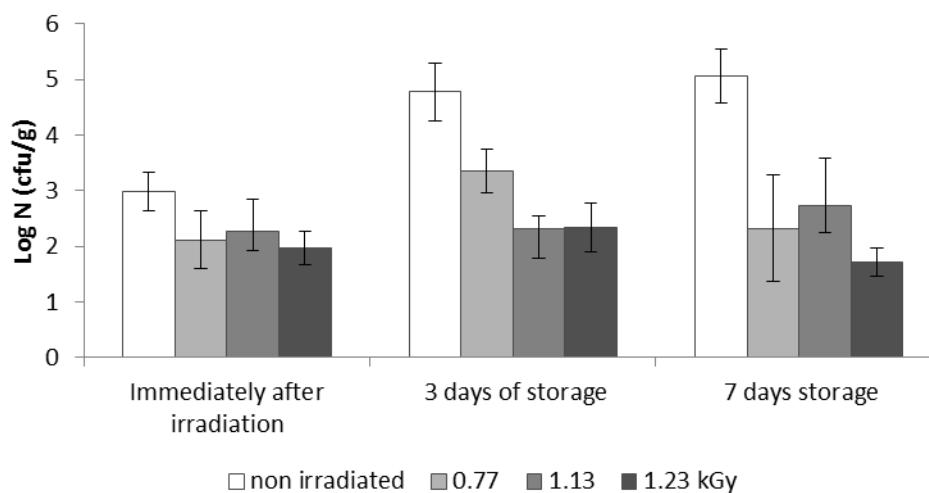
**Figure 12.** Total mesophilic microbial survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries of batches 1 and 2 (0.74 kGy light grey bar; 1.06 kGy grey bar and 1.58 kGy dark grey bar) for first sampling along storage time. Standard deviation bar bars correspond to 95% confidence intervals about mean values ( $n=3$ ;  $\alpha=0.05$ )

Regarding the fungal population (log CFU/g) of blackberries from Portugal (batch 1 and 2) the obtained results did not show an inactivation tendency for the applied gamma radiation doses (Figure 13), either immediately after irradiation or after seven days of storage.



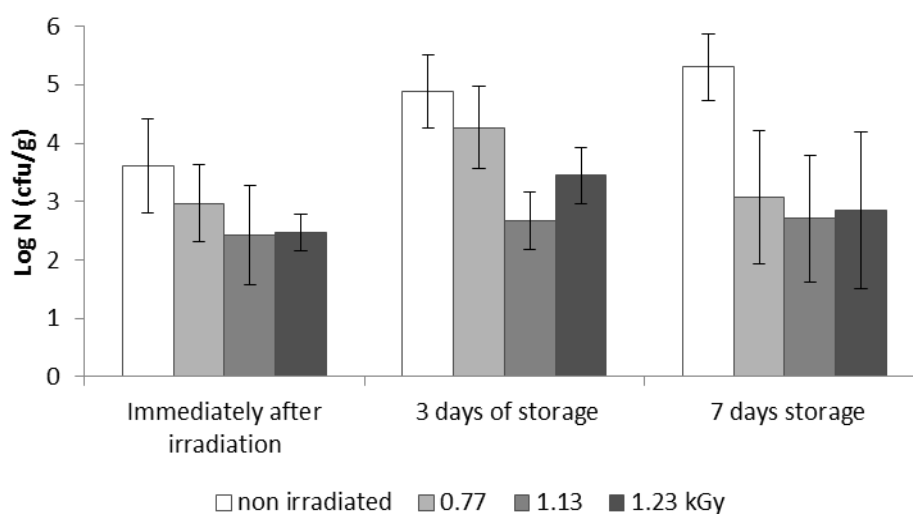
**Figure 13.** Total fungal population survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries for batches 1 and 2 (0.74 kGy light grey bar; 1.06 kGy grey bar and 1.58 kGy dark grey bar) of first sampling along storage time. Standard deviation bars correspond to 95% confidence intervals about mean values ( $n=3$ ;  $\alpha=0.05$ )

Results obtained with Netherlands blackberries (batch 3) point out to a decrease of bioburden with irradiation (Figure 14). The microbial inactivation efficiency was about 90% (1.0 log reduction), immediately after irradiation at 1.23 kGy. After three and seven days of storage it was verified an increase of the inactivation efficiency to > 99% for the applied dose of 1.23 kGy (2.43 and 3.35 log).



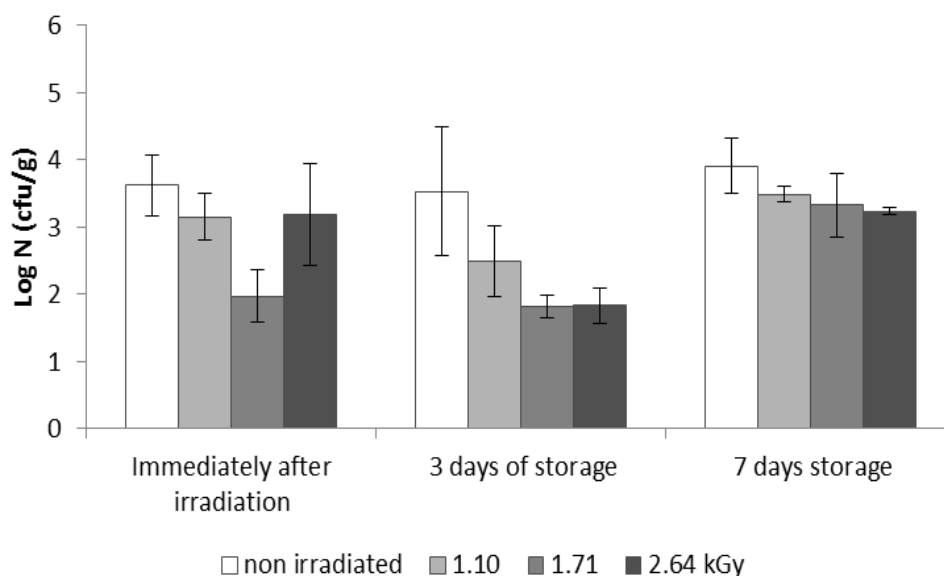
**Figure 14.** Total mesophilic microbial survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries of batch 3 (0.77 kGy light grey bar; 1.13 kGy grey bar and 1.23 kGy dark grey bar) of second sampling along storage time. Standard deviation bars correspond to 95% confidence interval about mean values ( $n=3$ ;  $\alpha=0.05$ ).

Concerning fungal population of the Netherlands blackberries (batch 3) it was also observed an increase of the inactivation efficiency with the irradiation doses along the storage time (> 97% for 1.23 kGy) (Figure 15).



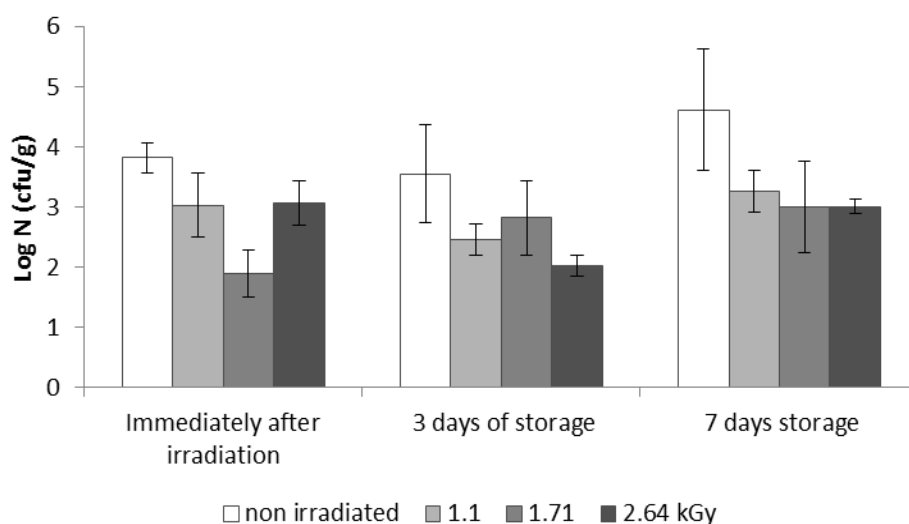
**Figure 15.** Total fungal population survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries for batch 3 (0.77 kGy light grey bar; 1.13 kGy grey bar and 1.23 kGy dark grey bar) of second sampling along storage time. Standard deviation bars correspond to 95% confidence intervals about mean values ( $n=3$ ;  $\alpha=0.05$ ).

For Mexican blackberries (Figure 16) a reduction of  $\leq 1.6$  log of microbial population was observed immediately after irradiation. Although, after three days of storage the microbiota reduction was maintained at 1.6 log. After the seven days under refrigerated conditions the blackberries microbial population reduction was below 0.8 log.



**Figure 16.** Total mesophilic microbial survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries of batch 4 (1.10 kGy light grey bar; 1.71 grey bar and 2.64 kGy dark grey bar) second sampling along storage time. Standard deviation bars correspond to 95% confidence interval about mean values ( $n=3$ ;  $\alpha=0.05$ ).

The fungal population of Mexican blackberries (batch 4) (Figure 17) was reduced by 0.7 up to 1.6 log, after irradiation and along storage.



**Figure 17.** Total fungal population survival (log CFU/g) of non-irradiated (white bar) and irradiated blackberries for batch 4 (1.10 kGy light grey bar; 1.71 grey bar and 2.64 kGy dark grey bar) of fourth batch, second sampling along storage time. Standard deviation bars correspond to 95% confidence intervals about mean values ( $n=3$ ;  $\alpha=0.05$ ).

Previous studies of gamma radiation on berries pointed out to limited microbial inactivation. For blueberry it was reported that inactivation of microbial load after irradiation, at doses between 0 up to 3 kGy (with intervals of 0.5 kGy) was reduced by approximately 1.5 log (Trigo *et al.*, 2006). Also, in raspberries, a reduction of 1 log in the microbial population was observed after irradiation at 1.5 kGy (Cabo Verde, 2013).

Several fungi were present and capable of growing on fresh fruits. Blackberries seem to be very susceptible to high levels of contamination. This circumstance is probably due to the fact that their skins are soft, easily ruptured with numerous indentation and hair-like protuberances which allow most organisms to attach and proliferate (Tournas, 2005).

The microbiota of non-irradiated and irradiated fruit were phenotypically characterized to evaluate the dynamics of blackberries microbial community and its pattern with radiation doses (Table 7).

The initial microbial population of blackberries from Portugal (batch 1 and 2) was mainly composed of filamentous fungi and yeasts during the seven days of storage. The natural microbiota of the blackberries from the batch 3 and 4 it revealed a different pattern, and presented as the most frequent morphotype the yeast and the gram negative oxidase positive rods. The blackberries from Mexico samples also demonstrated a significant frequency of filamentous fungi. During storage time, it was verified a decrease of the relative frequency of gram negative oxidase positive rods and an increase of endospore forming rods and fungi in Netherlands and Mexican blackberries, respectively.

A wide variety of fungi (mostly moulds) is capable of growing and spoiling various types of berries; considering the fact that these commodities contain high levels of sugar and other nutrients, a low pH and a water activity, ideal for fungal growth. A study of characterization of fungal population from small fruit indicated the presence of fungi in 100% of fungi in raspberries and blackberries samples, 97% of strawberries and 95% of blueberry samples. The same study refers to a lower incidence of yeast in berries, a result that has been partially explained taking into account the greater difficulty of such microorganisms to break the skin of these fruit and infected the internal tissues, unlike

the moulds (Tournas, 2005). Some of these moulds could produce mycotoxins while growing on fruits even during refrigeration (Tournas, 2001).

After irradiation, the Portuguese blackberries showed a higher frequency of yeast and filamentous fungi. Either immediately after irradiation or after two or seven days of storage it was noted an increasing tendency of the frequency of yeasts with the increase of the radiation dose (Table 7).

For the second blackberries sampling (blackberries from Netherlands and Mexico) similar results were observed, namely it is verified the same majority of fungi and yeasts after irradiation and an increase of yeasts with the increase of the radiation dose. An exception was for the Mexican blackberries that indicated after seven days of storage a decrease of the yeasts frequency and an increase of the frequency of filamentous fungi with increasing irradiation dose (Table 7). According to the literature, fungal spoilage of fruits will depend on cultivation, harvesting, handling, transport, and post-harvest storage and marketing conditions. Additionally, the low pH of these fruits eliminates the competition from many bacterial species, making it easier for fungi to grow and spoil the fruits (Tournas, 2005).

The microbial patterns obtained for the applied gamma irradiation doses could be explained considering the initial inactivation (at lower doses) of sensitive microorganisms, and maintenance of the viability of more resistant microorganisms with increasing radiation dose. The observed surviving microbial population in irradiated blackberries was homogeneous along time, with the prevalence of fungi and yeasts. A previous study on raspberries also indicated the predominance of filamentous fungi after irradiation at 1.5 kGy (Cabo Verde, 2013). For most of the fungi the lethal dose ranges from 5 to 10 kGy (International Atomic Energy Agency, 2008). The increase in the relative frequency of yeasts with the increased of the irradiation dose can be explained by the higher radiation resistance of this type of organisms compared to vegetative bacteria and fungi (Barth, 2009). The same results were mentioned in cherries exposed to gamma radiation (Pereira, 2012).

**Table 7.** Relative frequency of the morphological phenotypes of the isolates from non-irradiated and irradiated blackberries with storage time of blackberries from Portugal (n = 603 isolates from samples immediately after irradiation; n=165 isolates from samples with two days storage after irradiation; n=1061 isolates from samples with seven days storage after irradiation) from Netherland (n = 824 isolates from samples immediately after irradiation; n= 6212 isolates from samples with three days storage after irradiation; n= 32891 isolates from samples with seven days storage after irradiation), and from Mexico (n = 3349 isolates from samples immediately after irradiation; n= 1795 isolates from samples with three days storage after irradiation; n= 2069 isolates from samples with seven days storage after irradiation).

Phenotypical typification	% of total microbiota of blackberries from Portugal				% of total microbiota of blackberries from Netherland				% of total microbiota of blackberries from Mexico			
	Dose (kGy)				Dose (kGy)				Dose (kGy)			
	Non irradiated	0.74	1.06	1.58	Non irradiated	0.77	1.13	1.23	Non irradiated	1.10	1.71	2.64
<b>Immediately after irradiation</b>												
<i>gram</i> positive catalase positive cocci	9.2	15.5	0.0	0.0								
<i>gram</i> positive without endospore forming rods									19.0	0.0	0.0	0.0
<i>gram</i> positive catalase positive endospore forming rods	11.3	0.0	1.8	0.0								
<i>gram</i> positive catalase negative endospore forming rods					0.9	28.9	0.0	0.0				
<i>gram</i> negative oxidase positive rods	5.9	0.0	11.8	1.9	22.0	0.0	2.8	0.0	43.2	0.0	0.0	0.0
<i>gram</i> negative, oxidase negative	0.0	4.2	0.0	0.0	0.0	0.0	15.0	0.0	1.1	0.0	0.0	0.0

rods													
Yeast	16.0	2.8	62.3	52.8	76.2	66.6	32.7	71,63	15.8	70.87	0.0	91.7	
Fungi filamentous	57.9	77.5	24.7	45.3	0.9	4.6	49.5	28,37	38.3	29.1	100.0	8.3	
<b>2/3 days after storage</b>													
<i>gram</i> positive catalase negative endospore forming rods					0.0	0.5	0.0	0.4	0.3	0.0	0.0	0.0	
<i>gram</i> negative, oxidase positive rods					54.7	1.1	0.0	0.0	84.6	0.0	1.9	0.0	
Yeast	20.0	9.5	32.4	43.3	45.3	97.8	43.0	99.2	14.1	22.1	83.0	100.0	
Fungi filamentous	80.0	90.5	67.7	56.7	0.0	0.6	57.0	0.4	1.0	77.9	15.1	0.0	
<b>7 days after irradiation</b>													
<i>gram</i> positive catalase positive endospore forming rods	0.0	0.0	34.1	0.0									
<i>gram</i> positive catalase negative endospore forming rods					29.2	0.0	0.0	0.0	4.7	0.0	0.0	0.0	
<i>gram</i> negative oxidase positive rods	5.2	0.6	0.0	0.0	13.3	0.7	0.0	0.0	0.0	0.0	0.8	0.0	
<i>gram</i> negativo, oxidase negativa rods	5.9	11.5	12.4	11.5									
Yeast	17.0	74.5	33.0	61.5	57.5	98.0	98.9	99.9	62.7	0.0	0.7	7.3	
Fungi filamentous	71.9	13.5	20.4	26.9	0.0	1.4	1.1	0.1	32.7	100.0	98.6	92.7	

*Chapter 4 - Physical  
characterization of  
blackberries*

## 4.1 Physical parameter for quality assessment

### 4.1.1 Colour

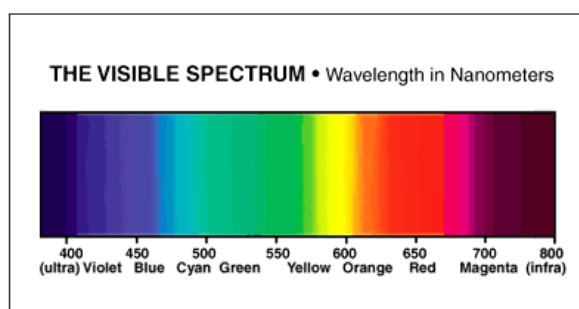
#### PRINCIPLES OF COLORIMETRY

The colour is an organoleptic characteristic and a very important quality attribute because it is easily understood by the consumer and is often associated with freshness, therefore, it is a major criterion in acceptance by consumers (Rodrigues, 2010).

The colour of a sample is subjectively evaluated when making an instrumental and visual sensorial evaluation (colorimeter for solid and semi-solid products and spectrophotometer for liquid products), and sometimes by chemical determination of the colorants (Lima, 2013).

The phenomenon of colour results from the physical interaction of a light source, a reflecting surface and an individual observer - three factors which can influence colour perception (Lima, 2013).

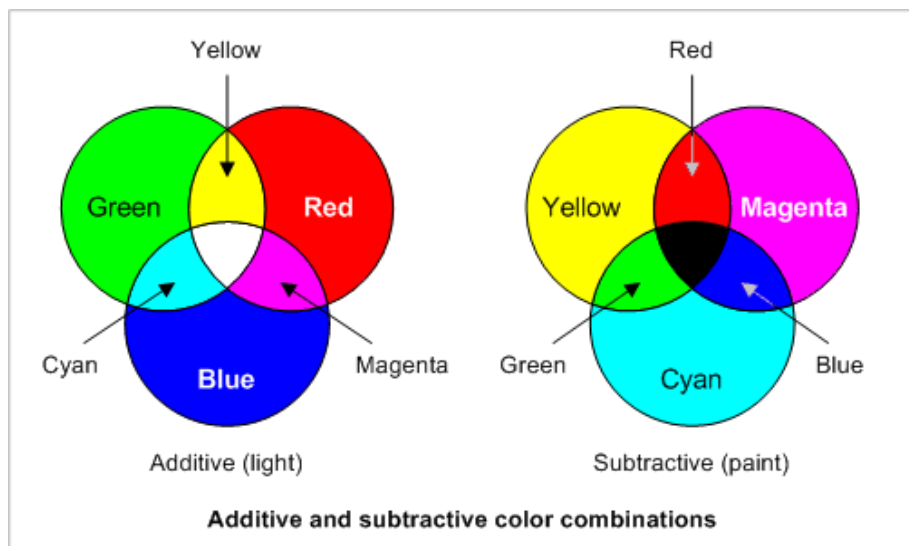
The light source is an object that emits radiant energy (light) sensitive to human eye. The emission is characterized by the relative amount of energy emitted at each wavelength of the visible spectrum (Figure 18) ([http://www.uff.br/fisicoquimica/docentes/katialeal/didatico/Capitulo\\_2.pdf](http://www.uff.br/fisicoquimica/docentes/katialeal/didatico/Capitulo_2.pdf)).



**Figure 18.** Wavelengths and visible spectrum (adapted from <http://www.aquaticcentral.com/plants/>)

Using the concepts of refraction and dispersion of light, Isaac Newton proved that white light when focused on a prism, decomposed in several wavelengths corresponding to the rainbow colours. Mixing different wavelengths of light (or colours) the white is obtained.

The additive primary colours are: Red, Green and Blue; theoretically the mixture of these three primary colours in the desired proportions will produce any colour. If these primary colours mixed in equal proportions, white light is obtained. When two primary colours are combined it will be obtained the subtractive primaries colours - Cyan, Yellow and Magenta (Figure 19). If combining these three subtractive colours in equal proportions, black is obtained, and if mixing two creates a secondary mixture of Red, Green and Blue (Scalco, 2003/2005).

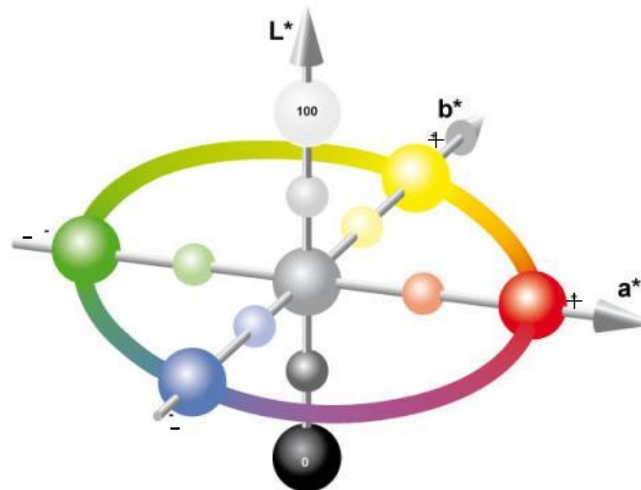


**Figure 19.** Additive and subtractive colour combinations (adapted from <http://socialdigital.dundee.ac.uk/~jastronska/blog/?p=399>)

Any material containing colour pigments or dyes can absorb, reflect or transmit light energy. An object that absorbs at a given wavelength will reflect energy at wavelengths opposed, for example, a yellow object will absorb blue energy and will reflect yellow energy ([http://dba.med.sc.edu/price/irf/Adobe\\_tg/models/rgbcmym.html](http://dba.med.sc.edu/price/irf/Adobe_tg/models/rgbcmym.html)).

## CIELAB system

The CIELab space (Figure 20), developed in 1976, supports the theory of colour perception based on three separate receivers RGB (Red, Green, Blue).



**Figure 20.** CIELab color space represents  $L^*a^*b^*$  color values in three dimensions (adapted from: <http://www.ern50.com/en/site/cielab-system>)

The CIELab space based on rectangular coordinates uses the axes  $L^*$ ,  $a^*$  and  $b^*$ , which define the three basic parameters in determining a colour specifically:

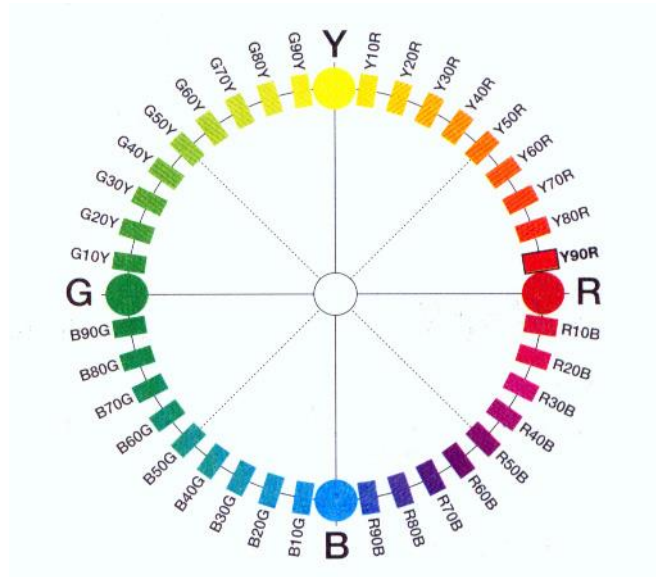
- $L^*$  - measures the variation of the brightness between black (0) up to white (100);
- $a^*$  - chromaticity coordinate, sets the colour red (positive values) up to green (negative values);
- $b^*$  - chromaticity coordinate, sets the colour yellow (positive values) up to blue (negative values).

When  $a^*$  and  $b^*$  approach zero, interpret neutral colours (white, grey and black).

The space  $CIEL^*C^*H^\circ$  describes cylindrical coordinates:

- $L^*$  - measures the variation of the brightness between black (0) and white (100);
- $C^*$  - corresponds to purity, giving us a value farthest from the origin according to the stronger and brighter the colour appears;
- $H^\circ$  - measure Hue and is represented by an angle from  $0^\circ$  to  $360^\circ$ . Angles between  $0^\circ$  and  $90^\circ$  are represented by the reds, oranges and yellows;  $90^\circ$  to  $180^\circ$  are yellow, yellow-green and green;  $180^\circ$  to  $270^\circ$  are green, cyan (blue -

green) and blue; 270° to 360° are blues, purples, magentas and reds again. A value of  $H^*$  equal to 360° is treated similarly to 0° (Figure 21) (Lima, 2013).



**Figure 21.** Represents  $H^\circ$  (hue) colour values (adapted from: <http://www.wetcanvas.com/forums>)

## COLORIMETER

The colorimeter measures the coordinates of several colour measurement systems. Uses standard illuminates, simulating day light - D65 (includes UV region) and C (without the UV region), A (incandescent light) (Figure 22).



**Figure 22.** Illustrative example of a colorimeter

From the values obtained from the *software* of the colorimeter ( $L^*$ ,  $a^*$  and  $b^*$ ) it is determined the Chroma ( $C^*$  - Equation 3), which will be stronger farther away from the origin of coordinates, and determined the hue ( $H^\circ$  - Equation 4):

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

**Equation 3.** Equation for the calculation of Chroma

$$H^\circ = \tan^{-1} \left( \frac{b^*}{a^*} \right)$$

**Equation 4.** Equation for the calculation of tonality

Where:  $a^* > 0$ ;  $b^* > 0$

For determining the total colour difference between two samples the equation of Adams-Nickerson is used (Equation 5):

$$TDC = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

**Equation 5.** Equation for the calculation of total colour difference

Where:  $TDC$  is equal to the total colour difference,  $\Delta L^*$  corresponds to the difference in light/dark,  $\Delta a^*$  the difference in red/green and  $\Delta b^*$  the difference in yellow/blue (Lima, 2013).

### 4.1.2 Total Soluble Solids Content - °Brix

The main quantitative transformation that occurs in fruit ripening is the conversion of starch into soluble sugars. This transformation affects the taste and texture of the fruit (<http://www.cpact.embrapa.br/publicacoes/documentos/documento-271.pdf>).

#### PRINCIPLES OF TOTAL SOLUBLE SOLIDS CONTENT - °BRIX

The light refraction is an optical phenomenon that happens when there is a change in the direction of a light beam as it passes through two transparent media with different densities. This angle varies with the concentration of the solution. The change of this angle (refraction angle), in degrees, indicating the change of direction of the light beam, is transformed in values of refractive index. This is a physical property and can be used

for the analysis of solids, liquids and gases (Moraes, 2006). The soluble solids content is used as an indirect measure of the sugar content, which is responsible for 85-90% of soluble solids (<http://www.cpact.embrapa.br/publicacoes/documentos/documento-271.pdf>).

The °Brix is the percentage of soluble solids in the juice of fruits and vegetables (<http://www.healthy-vegetable-gardening.com/brix-level.html>). When measuring the refractive index of a sucrose solution, reading indicates the °Brix value which corresponds to the sucrose concentration in this aqueous solution

The °Brix scale is calibrated with solutions of sucrose of different percentages. The reading of the measured value is the amount of solids dissolved in water (sugar, minerals, and proteins) (Moraes, 2006). For the consumer, a higher °Brix level means a better flavour (<http://www.healthy-vegetable-gardening.com/brix-level.html>).

### REFRACTOMETER ABBÉ

The principle of measurement for the refractometer Abbé (Figure 23) is through the register of threshold angle of the total reflection. This device can measure by two different methods: i) transmission of the incident light; ii) reflection (<http://sites.ifi.unicamp.br/lf22/15-refratometria-abbe-e-pulfrich/>). The equipment obtains and turns the refractive angles on values of indices of refraction (Moraes, 2006).



**Figure 23.** Refractometer abbé

The refractometer was developed for several purposes: determining the refraction index, concentration, purity and dispersion of liquid samples, among others. It has applicability in aqueous, alcoholic and ethereal solutions, oils, waxes, syrups, food, among others. The values can be read in refractive index or °Brix (<http://www.prolab.com.br/produtos/equipamentos-para-laboratorio/refratometro-de-bancada--abbe>).

It is necessary to calibrate before measurements. Distilled water is normally used at a studied temperature or a standard supplied with the equipment to verify that the setting is correct (<http://sites.ifi.unicamp.br/lf22/15-refratometria-abbe-e-pulfrich/>). The refractive index of water at 20 ° C is 1.3333 (Moraes, 2006).

### *4.1.3 Texture*

#### **PRINCIPLES OF TEXTURE**

Texture is a sensory acceptability factor, since it is directly perceived by the senses. According to ISO 11036:1994 the texture is all mechanical, geometrical and surface attributes of a product, detectable by mechanical, tactile and when appropriate for visual and auditory receptors.

The texture of foods is a property of difficult definition that comprises a set of physical properties with sensory implications depending on the structure of the food, which is determined by its chemical composition. This feature can be evaluated by the sense of touch in the hands or mouth, by sight and hearing as is the case with crispy french fries or toast and has a strong cultural component when evaluated sensorially (Sousa, 2001).

The main principle used on instrumental determination of texture is the contact of the sample with a probe. This is deformed and the degree of deformation and/or resistance offered is logged and used as an index of food texture (Lima, 2011; Barradas, 1999).

The importance of texture quality of the food product varies widely according to the type of product, being or not, considered as a critical factor (Bourne, 2002).

## TEXTUROMETER STEVENS QTS - 25

The texture can be measured by several instruments such as the penetrometer (e.g. firmness of butter/margarine) compressmeter (e.g. aging of bread), tenderometer (e.g. shear strength the muscle fibres of the meat) extrusimeter (e.g. jams texture), texturometer (depending on the rheological material under study) and other non-destructive (Lima, 2011).

The texturometer Stevens QTS - 25 (Figure 24) is a bench top and auto-sufficient instrument, providing accuracy analysis of products even in production environments and is accompanied by the TexturePro v 2.0 Software.



**Figure 24.** Texturometer Stevens QTS - 25

The device consists of an arm which moves on vertically where is adapted the appropriate probe to the desired test (e.g. compression, penetration, cutting, extrusion). It has a dynamometer which provides mechanical energy at constant rate. The result is a curve of force versus time or force versus distance where is related the change in the texture of the material (Castro *et al.*, 2001).

Same probes can be used in different tests, taking into account the desired parameters and food in analysis (e.g. cylindrical, conical, spherical or needle).

## 4.2 Methodology of physical analysis

### 4.2.1 Colour methodology

Colour of blackberries was assessed with a Colorimeter KONICA MINOLTA CR 400. Ten measurements were performed for each irradiated and non-irradiated samples (ten blackberries from each sample) and data was analysed using Spectra Magic Nx Software (Figure 25).

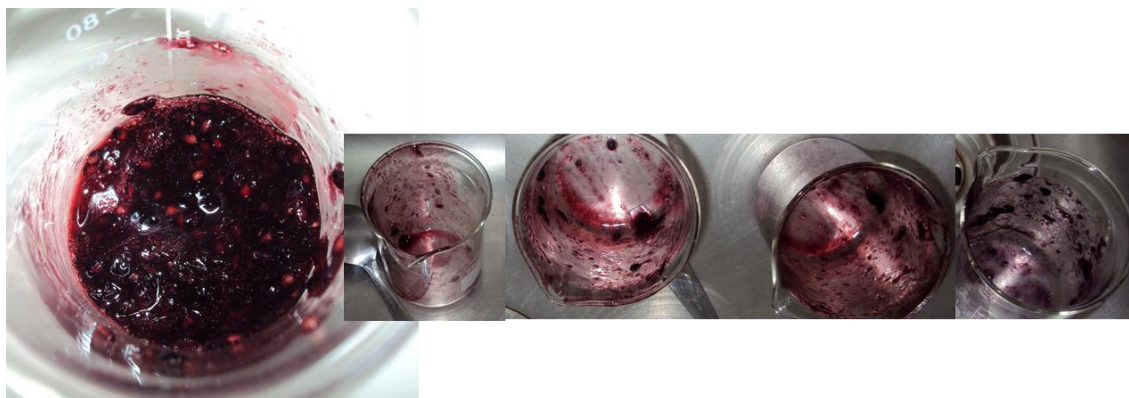


**Figure 25.** Illustrative example of the colour analysis of blackberries

From the obtained values ( $L^*$ ,  $a^*$  and  $b^*$ ), the Chroma ( $C^*$ ) and Hue ( $H^\circ$ ) values were determined. The methodology was the same for each of the two samplings analysed.

### 4.2.2 Total soluble solids methodology

The total soluble solids (TSS) content was assessed with a refractometer ABBÉ. The equipment was previously calibrated at 24°C, with distillate water. Five blackberries per dose were squeezed until juice was obtained for analysis (Figure 26). Four measurements were done per juice sample and results were expressed as °Brix.



**Figure 26.** Juice obtained for analysis and different colour of juice from all samples of blackberries from Mexico (increase of doses from left to right – 0, 1.10 kGy, 1.71 kGy, 2.64 kGy)

### 4.2.3 *Texture methodology*

The texture of blackberries under study was analysed using a texturometer Stevens QTS-25 controlled from a console or by the computer (Figure 27).



**Figure 27.** Illustrative example of the texture analysis of blackberries

The test carried out on the analysis of blackberries was a puncture test. The following texture parameters were measured: i) fracture force; ii) firmness; iii) modulus apparent; iv) adhesive force; v) adhesiveness; vi) peak load; and vii) deformation of peak load. The test conditions are presented in Table 8.

**Table 8.** Test conditions for rehearsals of the texture of blackberries

<i>Rehearsals conditions</i>	
<b>Target test</b>	Puncture
<b>Probe type</b>	Needle
<b>“Trigger point”</b>	5g
<b>Thickness probe</b>	2mm
<b>Test speed</b>	80mm/min
<b>Number of cycles</b>	1
<b>Hold time</b>	0s
<b>Target Unit</b>	Distance
<b>Target Value</b>	8mm
<b>Test number</b>	10blackberries/sample

Some evaluation parameters were selected and its definition is in Table 9 (adapted from Current Protocols in Food Analytical Chemistry, 2001).

**Table 9.** Parameters selected for the analysis of the texture of blackberries

<i>Parameters</i>	<i>Unidades</i>	<i>Definição</i>
Fracture force	G	Force required to fracture the material
Rigidity	g/s	Deformation caused by force
Firmness	G	Compression or penetration force required to achieve the desired deformation (hard, soft)
Adhesive force	G	Maximum force of attraction of food
Adhesiveness	g.s	Work required to overcome the forces of attraction of the surface of the food (sticky adhesive)

## 4.2.4 *Statistical Analysis*

The STATISTICA 9.1 program was used for the statistical treatment of the results values has been tested by the significance of Wilks test in order to be able to use the ANOVA analysis. The post-hoc Fisher's LSD mean comparison test was used to evaluate the differences between the samples for a confidence interval of 95%.

## 4.3 *Results and discussion of physical analysis*

### 4.3.1 *Colour assessment*

The surface colour is one of the most appealing factors that influence consumers when buying fruit. Therefore evaluation of the global colour properties was done for irradiated and non-irradiated blackberry fruits (Table 10 – first sampling (blackberries from Portugal; Table 11 – third batch, second sampling (blackberries from Netherland); Table 12 – fourth batch, second sampling (blackberries from Mexico), respectively).

For blackberries from Portugal, no significant differences were observed in brightness between non-irradiated and irradiated blackberries, immediately after irradiation or after storage of two or seven days (Table 10). It was found, that in the case of blackberries with seven days of storage, the colour is even less pure than two days of storage. Hue value was lower in blackberries immediately after irradiation and two days of storage, indicating a higher tendency for red, compared to non-irradiated samples. However, these results were not observed between samples stored for seven days, where no significant differences were found between non-irradiated and irradiated samples. The blackberries irradiated at 1.06 kGy after two days of storage presented the lowest value, however the highest Hue value obtained for the same applied dose and after seven days of storage. A decrease in hue values with storage was also observed.

**Table 10.** Colour parameters for non-irradiated and irradiated blackberry fruits for the first sampling (blackberries from Portugal). Mean values  $\pm$  SD (standard deviation) obtained for the colorimetric parameters for non-irradiated and irradiated (n=10) samples.

	<b>L*</b>	<b>C*</b>	<b>H°</b>
	<b><math>\pm</math>SD</b>	<b><math>\pm</math>SD</b>	<b><math>\pm</math>SD</b>
<b>Immediately after irradiation</b>			
Non irradiated	18.93 <sup>ab</sup> $\pm$ 1.00	1.25 <sup>a</sup> $\pm$ 0.36	28.09 <sup>e</sup> $\pm$ 15.30
0.74 kGy	20.36 <sup>d</sup> $\pm$ 0.86	1.11 <sup>a</sup> $\pm$ 0.29	18.65 <sup>ab</sup> $\pm$ 9.92
1.06 kGy	19.24 <sup>abc</sup> $\pm$ 1.31	1.28 <sup>a</sup> $\pm$ 0.27	22.55 <sup>b</sup> $\pm$ 6.87
1.58 kGy	19.67 <sup>acd</sup> $\pm$ 1.10	1.33 <sup>a</sup> $\pm$ 0.36	17.24 <sup>ab</sup> $\pm$ 8.65
<b>2 days of storage</b>			
Non irradiated	17.51 <sup>tg</sup> $\pm$ 1.05	1.42 <sup>a</sup> $\pm$ 0.35	16.59 <sup>ab</sup> $\pm$ 5.75
0.74 kGy	18.72 <sup>ab</sup> $\pm$ 1.46	1.38 <sup>a</sup> $\pm$ 0.30	16.21 <sup>abd</sup> $\pm$ 7.51
1.06 kGy	16.99 <sup>f</sup> $\pm$ 0.63	1.48 <sup>a</sup> $\pm$ 0.51	7.52 <sup>c</sup> $\pm$ 2.13
1.58 kGy	18.58 <sup>b</sup> $\pm$ 1.17	1.33 <sup>a</sup> $\pm$ 0.44	9.57 <sup>cd</sup> $\pm$ 6.51
<b>7 days of storage</b>			
Non irradiated	18.17 <sup>e</sup> $\pm$ 0.82	2.36 <sup>b</sup> $\pm$ 1.42	13.32 <sup>abd</sup> $\pm$ 3.76
0.74 kGy	19.38 <sup>abcd</sup> $\pm$ 1.36	3.17 <sup>b</sup> $\pm$ 2.24	14.08 <sup>abd</sup> $\pm$ 7.10
1.06 kGy	19.62 <sup>acd</sup> $\pm$ 1.23	2.57 <sup>b</sup> $\pm$ 1.07	19.90 <sup>ab</sup> $\pm$ 7.48
1.58 kGy	20.13 <sup>cd</sup> $\pm$ 1.36	2.36 <sup>b</sup> $\pm$ 0.99	13.23 <sup>abd</sup> $\pm$ 3.90

L\*- brightness; C\*- chroma; H° - hue. For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ).

For blackberries from Netherland, no significant differences in brightness and hue values between non-irradiated and irradiated blackberries were observed immediately after irradiation or after storage of three or seven days (Table 11). The blackberries with three and seven days of storage showed a decrease of hue with increase dose of irradiation. Chroma was higher in non-irradiated blackberries immediately after irradiation. Some differences in values of Chroma were observed, although they didn't show a trend.

**Table 11.** Colour parameters for non-irradiated and irradiated blackberry fruits for the first batch of second sampling (blackberries from Netherland). Mean values  $\pm$  SD (standard deviation) obtained for the colorimetric parameters for non-irradiated and irradiated (n=10) samples.

	<b>L*</b>	<b>C*</b>	<b>H°</b>
	<b><math>\pm</math>SD</b>	<b><math>\pm</math>SD</b>	<b><math>\pm</math>SD</b>
<b>Immediately after irradiation</b>			
Non irradiated	18.50 <sup>ab</sup> $\pm$ 1.70	4.18 <sup>a</sup> $\pm$ 3.58	32.80 <sup>bc</sup> $\pm$ 7.58
0.77 kGy	19.08 <sup>b</sup> $\pm$ 1.76	2.95 <sup>c</sup> $\pm$ 0.56	35.05 <sup>c</sup> $\pm$ 7.88
1.13 kGy	18.14 <sup>ab</sup> $\pm$ 1.77	4.12 <sup>ab</sup> $\pm$ 0.85	27.50 <sup>a</sup> $\pm$ 4.40
1.23 kGy	18.50 <sup>ab</sup> $\pm$ 0.93	2.84 <sup>c</sup> $\pm$ 0.49	29.43 <sup>ab</sup> $\pm$ 6.86
<b>3 days of storage</b>			
Non irradiated	18.91 <sup>ab</sup> $\pm$ 1.55	3.14 <sup>bc</sup> $\pm$ 0.37	29.18 <sup>ab</sup> $\pm$ 4.10
0.77 kGy	19.10 <sup>b</sup> $\pm$ 0.85	3.05 <sup>c</sup> $\pm$ $\pm$ 0.47	30.71 <sup>abc</sup> $\pm$ 5.67
1.13 kGy	18.65 <sup>ab</sup> $\pm$ $\pm$ 1.64	3.02 <sup>c</sup> $\pm$ $\pm$ 0.37	29.09 <sup>ab</sup> $\pm$ 5.16
1.23 kGy	17.76 <sup>a</sup> $\pm$ 1.83	3.23 <sup>bc</sup> 0.54	25.93 <sup>a</sup> $\pm$ 4.13
<b>7 days of storage</b>			
Non irradiated	18.40 <sup>ab</sup> $\pm$ 0.89	2.93 <sup>c</sup> $\pm$ 0.38	35.19 <sup>c</sup> $\pm$ 5.16

0.77 kGy	18.27 <sup>ab</sup> ± 1.35	3.14 <sup>bc</sup> ± 0.41	29.89 <sup>ab</sup> ± 6.84
1.13 kGy	18.14 <sup>ab</sup> ± 1.18	2.86 <sup>c</sup> ± 0.33	29.20 <sup>ab</sup> ± 5.91
1.23 kGy	17.76 <sup>a</sup> ± 1.83	3.23 <sup>bc</sup> ± 0.54	25.93 <sup>a</sup> ± 4.13

L\*- brightness; C\*- chroma; H° - hue. For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ).

For blackberries from Mexico a decrease of brightness was observed with the increase of radiation dose immediately after irradiation and seven days of storage (Table 12). The blackberries immediately after irradiation showed an increase of purity of colour with irradiation, and the blackberries irradiated at 2.64 kGy exhibited the higher value. No significant differences were observed in hue values between non-irradiated and irradiated blackberries, immediately after irradiation. The blackberries with two and seven days of storage showed some differences in values of hue but did not follow any trend.

**Table 12.** Colour parameters for non-irradiated and irradiated blackberry fruits for the second batch of second sampling (blackberries from Mexico). Mean values ± SD (standard deviation) obtained for the colorimetric parameters for non-irradiated and irradiated (n=10) samples

	<b>L*</b> ±SD	<b>C*</b> ±SD	<b>H°</b> ±SD
<b>Immediately after irradiation</b>			
Non irradiated	19.07 <sup>cd</sup> ± 0.91	1.83 <sup>a</sup> ±0.20	15.31 <sup>ab</sup> ±6.57
1.10 kGy	17.73 <sup>ab</sup> ± 0.73	2.20 <sup>ab</sup> ± 0.32	13.86 <sup>ab</sup> ± 4.87
1.71 kGy	17.33 <sup>a</sup> ±1.81	2.48 <sup>bc</sup> ±0.36	14.02 <sup>ab</sup> ±4.00
2.64 kGy	17.99 <sup>ab</sup> ±1.45	2.81 <sup>cd</sup> ±0.46	16.48 <sup>b</sup> ±2.46
<b>3 days of storage</b>			
Non irradiated	19.55 <sup>ce</sup> ±1.05	2.78 <sup>cd</sup> ± 0.88	15.60 <sup>ab</sup> ±2.88

1.10 kGy	20.18 <sup>e</sup> ± 0.85	2.47 <sup>bc</sup> ±0.32	11.53 <sup>a</sup> ± 1.99
1.71 kGy	20.19 <sup>e</sup> ± ±0.78	2.56 <sup>bc</sup> ±0.33	16.79 <sup>bcd</sup> ±5.28
2.64 kGy	19.07 <sup>cd</sup> ±1.21	3.13 <sup>d</sup> ±0.53	19.86 <sup>cdef</sup> ±4.41
<b>7 days of storage</b>			
Non irradiated	19.04 <sup>cd</sup> ± 1.43	3.08 <sup>d</sup> ± 0.37	17.44 <sup>bcd</sup> ±3.60
1.10 kGy	19.27 <sup>cde</sup> ± 0.97	2.98 <sup>d</sup> ± 0.26	21.26 <sup>ef</sup> ± 5.86
1.71 kGy	18.52 <sup>bcd</sup> ± 1.45	3.09 <sup>d</sup> ± 0.49	22.72 <sup>f</sup> ± 4.93
2.64 kGy	18.36 <sup>abd</sup> ± 0.80	3.15 <sup>d</sup> ±0.50	20.87 <sup>def</sup> ± 6.13

L\* - brightness; C\* - chroma; H° - hue. For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ).

According to other authors the hue angle decreases in storage due to anthocyanin synthesis, responsible for the red colour on berry fruits (Han, 2004). A previous study reported that irradiation at doses between 0.5 and 1.5 kGy did not have a pronounced effect on the colour of raspberries. However, the authors also found a decrease in colour parameters after two days storage in both irradiated and non-irradiated fruit (Cabo Verde, 2013). A study with strawberries, reported that gamma irradiation increased the rate of change of fruit colour from green to red (Al-Bachir, 1999). In *Nelumbo nucifera* was reported that  $\gamma$ -irradiation significantly increased the L\* value until doses of 20 and 50 kGy, and the b\* values were decreased under the same conditions (Jeong, 2009).

### 4.3.2 *Total soluble solids assessment*

Other important fruit quality parameter is TSS content. Therefore evaluation of the °brix value was performed for irradiated and non-irradiated blackberry fruits (Table 13 for all batches). The mean values of TSS of non-irradiated samples for blackberries from Portugal ( $9.22 \pm 0.06$ ) were slightly higher than those found in the literature ( $\approx 8.0^\circ\text{Brix}$ ) (Raseira, 2008). For second sampling, the blackberries from Netherlands showed a value slightly below the literature ( $7.31 \pm 0.13$ ), however the Mexican blackberries showed a value significantly higher °brix ( $12.44 \pm 0.13$ ). Nevertheless, it has been described in literature that tropical highland blackberries exhibit noticeably higher acidity and much lower soluble solids content than do blackberry cultivars grown in temperate climates (Acosta-Montoya, 2009).

According to literature, depending on the cultivar, soluble solids content may increase from approximately 4 to 15.7% from red fruit to black fruit. In black fruits, soluble solids may still enhance approximately 10–18%. Blackberries contain about 5 to 6% total sugars, from which 1 to 5% is sucrose, 44 to 48% is glucose, and 47 to 49% is fructose (Nunes, 2008).

Mean values of TSS for irradiated and non-irradiated blackberries from the first sampling (blackberries from Portugal) are shown in Table 13. Results show that immediately after irradiation, TSS decreased with irradiation. After two days of storage no significant changes occurred in this parameter for non-irradiated blackberry fruits and irradiated sample at 1.58 kGy decreased slightly. Only an increase was found for samples irradiated with 0.74 kGy and 1.06 kGy. For seven days of storage TSS values showed a marked decrease in the non-irradiated and irradiated blackberries at 0.74 kGy, however at higher doses the values were maintained.

For third batch (blackberries from Netherlands) a slight increase of TSS was observed with the increase of the irradiation dose, immediately after irradiation and three days of storage (Table 13). After seven days of storage there was observed a decrease of TSS for irradiated and non-irradiated blackberries. At three days of storage the TSS values were higher than the other evaluated times.

For the fourth batch of blackberries (from Mexico), the TSS values indicated no significant differences between the non-irradiated and irradiated fruits at the highest dose (2.64 kGy), immediately after irradiation and after seven days of storage (Table 13). After three days of storage, it was noticed a considerable increase in °Brix ( $\approx 2$ ) for the higher dose compared to non-irradiated sample.

**Table 13.** Totals soluble solids (TSS) of non-irradiated and irradiated blackberries fruits, mean (n=4) of the all batches.

TSS (°brix) at 24°C (Mean±DP)					
Blackberries from	Portugal (batch 1 and 2)		Netherlands (batch 3)		Mexico (batch 4)
<b>Immediately after irradiation</b>					
Non irradiated	9.22 <sup>e</sup> ± 0.06		7.31 <sup>a</sup> ± 0.13		12.44 <sup>d</sup> ± 0.13
0.74 kGy	7.75 <sup>b</sup> ± 0.00	0.77 kGy	8.44 <sup>c</sup> ± 0.13	1.10 kGy	10.25 <sup>a</sup> ± 0.20
1.06 kGy	8.81 <sup>d</sup> ± 0.07	1.13 kGy	8.75 <sup>b</sup> ± 0.00	1.71 kGy	12.50 <sup>d</sup> ± 0.20
1.58 kGy	8.44 <sup>c</sup> ± 0.13	1.23 kGy	8.81 <sup>d</sup> ± 0.24	2.64 kGy	12.56 <sup>d</sup> ± 0.13
<b>2 days of storage</b>		<b>3 days of storage</b>			
Non irradiated	9.13 <sup>e</sup> ± 0.14		8.50 <sup>c</sup> ± 0.00		11.25 <sup>b</sup> ± 0.20
0.74 kGy	10.00 <sup>f</sup> ± 0.00	0.77 kGy	9.31 <sup>e</sup> ± 0.13	1.10 kGy	11.94 <sup>c</sup> ± 0.13
1.06 kGy	10.00 <sup>f</sup> ± 0.00	1.13 kGy	9.63 <sup>f</sup> ± 0.14	1.71 kGy	12.88 <sup>e</sup> ± 0.14
1.58 kGy	8.81 <sup>d</sup> ± 0.13	1.23 kGy	9.94 <sup>g</sup> ± 0.13	2.64 kGy	13.13 <sup>f</sup> ± 0.14
<b>7 days of storage</b>					
Non irradiated	7.06 <sup>a</sup> ± 0.13		7.94 <sup>b</sup> ± 0.13		12.81 <sup>e</sup> ± 0.13
0.74 kGy	7.13 <sup>a</sup> ± 0.14	0.77 kGy	8.31 <sup>c</sup> ± 0.24	1.10 kGy	11.75 <sup>c</sup> ± 0.20
1.06 kGy	9.94 <sup>f</sup> ± 0.24	1.13 kGy	8.75 <sup>d</sup> ± 0.20	1.71 kGy	11.31 <sup>b</sup> ± 0.13
1.58 kGy	8.81 <sup>d</sup> ± 0.13	1.23 kGy	7.75 <sup>b</sup> ± 0.29	2.64 kGy	12.94 <sup>ef</sup> ± 0.13

The values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ) for samples from the same batch.

The observed differences between the three samplings in study can be explained considering the variances of fruits maturity degree there was a large time dissimilarity among harvest and sale for each of the three samples.

According to other authors the TSS of blackberries do not change during storage, suggesting that the increase in sugar content may be the result of the evolution of ripeness, balanced with the breath of the fruit (Wu, 2010). A study on effect of gamma irradiation on quality of kiwifruit (0; 1; 2 and 3 kGy) indicated a decrease in the total soluble solids content with increasing irradiation dose (Kim, 2009). For seeds of anise exposed to gamma radiation doses of 5 kGy, 10 kGy and 20 kGy it was observed that immediately after irradiation, the TSS content of irradiated seeds were higher than those of non-irradiated (Al-Bachir, 2005).

### *4.3.3 Texture assessment*

Texture is another critical factor to consumer acceptance of a product. The term “firmness of fruit” is commonly used to describe a parameter assessed by means of empirical mechanical tests and understood as an attribute that ought to be maintained during storage and processing. Firmness, interpreted as a mechanical response intrinsic to the fruit structure, is influenced by the stage of physiological development, degree of ripeness, damage, fibrousness and turgidity (Sousa, 2007).

The results of the texture analysis of the first blackberry sampling are presented in Table 14. No significant differences were observed in both fracturability and firmness between non-irradiated and irradiated blackberries immediately after irradiation. In blackberries stored for two days, both parameters were slightly lower in irradiated blackberries, compared to non-irradiated blackberries.

Regarding rigidity no significant differences were detected between non-irradiated and irradiated blackberries (Table 14), either immediately after irradiation or after two days storage. Adhesive force was higher in blackberries immediately after irradiation at 1.06 kGy, than in non-irradiated samples. However, these results were not observed for samples stored for two days, where no significant differences were found between non-irradiated and irradiated samples.

**Table 14.** Texture parameters for non-irradiated and irradiated blackberry fruits from Portugal (first sampling). Average values  $\pm$  SD for texture parameters of the samples in study (n=10).

	Fracturability $\pm$ SD	Firmness $\pm$ SD	Rigidity $\pm$ SD	Adhesive Force $\pm$ SD
<b>Immediately after irradiation</b>				
<b>Non irradiated</b>	0,09 <sup>ab</sup> $\pm$ 0,08	0,13 <sup>a</sup> $\pm$ 0,15	0,80 <sup>a</sup> $\pm$ 0,44	0,05 <sup>ab</sup> $\pm$ 0,04
<b>0.74 kGy</b>	0,11 <sup>ab</sup> $\pm$ 0,07	0,23 <sup>ab</sup> $\pm$ 0,09	0,58 <sup>a</sup> $\pm$ 0,22	0,05 <sup>ab</sup> $\pm$ 0,03
<b>1.06 kGy</b>	0,09 <sup>ab</sup> $\pm$ 0,05	0,22 <sup>a</sup> $\pm$ 0,10	0,58 <sup>a</sup> $\pm$ 0,63	0,16 <sup>c</sup> $\pm$ 0,06
<b>1.58 kGy</b>	0,08 <sup>ab</sup> $\pm$ 0,12	0,15 <sup>a</sup> $\pm$ 0,12	0,20 <sup>a</sup> $\pm$ 0,37	0,08 <sup>b</sup> $\pm$ 0,07
<b>2 days of storage</b>				
<b>Non irradiated</b>	0,22 <sup>b</sup> $\pm$ 0,15	0,41 <sup>b</sup> $\pm$ 0,18	0,33 <sup>a</sup> $\pm$ 0,17	0,03 <sup>ab</sup> $\pm$ 0,02
<b>0.74 kGy</b>	0,10 <sup>ab</sup> $\pm$ 0,08	0,21 <sup>a</sup> $\pm$ 0,09	0,51 <sup>a</sup> $\pm$ 0,23	0,022 <sup>ab</sup> $\pm$ 0,01
<b>1.06 kGy</b>	0,07 <sup>a</sup> $\pm$ 0,05	0,17 <sup>a</sup> $\pm$ 0,05	0,44 <sup>a</sup> $\pm$ 0,37	0,02 <sup>a</sup> $\pm$ 0,02
<b>1.58 kGy</b>	0,07 <sup>a</sup> $\pm$ 0,03	0,21 <sup>a</sup> $\pm$ 0,11	0,65 <sup>a</sup> $\pm$ 0,50	0,03 <sup>ab</sup> $\pm$ 0,02

The results are reported as force in Newton (N). For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $P > 0.05$ ).

The texture parameters results of the blackberries from Netherland (third batch) are shown in Table 15. No significant differences were observed in both fracturability and rigidity between non-irradiated and irradiated blackberries for the analysed storage times. The firmness of Netherlands blackberries pointed out to a decrease over storage time in storage. For this parameter, the non-irradiated sample has the highest value compared with the irradiated blackberries. The adhesive force of blackberries seems to decrease during storage time from non-irradiated and irradiated samples at 0.77 kGy and 1.13 kGy. However, the irradiated fruits at 1.23 kGy indicated the opposite

behavior. Any trends according to the irradiation dose for the parameters analyzed in this batch were not verified.

**Table 15.** Texture parameters of non-irradiated and irradiated blackberry fruits from Netherland (third batch, second sampling). Average values  $\pm$  SD for texture parameters of the samples in study (n=10).

	Fracturability $\pm$ SD	Firmness $\pm$ SD	Rigidity $\pm$ SD	Adhesive Force $\pm$ SD
<b>Immediately after irradiation</b>				
<b>Non irradiated</b>	0.11 <sup>abc</sup> $\pm$ 0.04	0.34 <sup>a</sup> $\pm$ 0.20	0.27 <sup>ab</sup> $\pm$ 0.23	0.07 <sup>ab</sup> $\pm$ 0.03
<b>0.77 kGy</b>	0.11 <sup>abc</sup> $\pm$ 0.07	0.27 <sup>abc</sup> $\pm$ 0.16	0.53 <sup>ab</sup> $\pm$ 0.47	0.07 <sup>a</sup> $\pm$ 0.02
<b>1.13 kGy</b>	0.08 <sup>ab</sup> $\pm$ 0.05	0.20 <sup>bc</sup> $\pm$ 0.16	0.40 <sup>ab</sup> $\pm$ 0.04	0.07 <sup>abc</sup> $\pm$ 0.02
<b>1.23 kGy</b>	0.12 <sup>abc</sup> $\pm$ 0.11	0.20 <sup>bc</sup> $\pm$ 0.10	0.26 <sup>ab</sup> $\pm$ 0.18	0.05 <sup>bcd</sup> $\pm$ 0.02
<b>3 days of storage</b>				
<b>Non irradiated</b>	0.08 <sup>abc</sup> $\pm$ 0.02	0.29 <sup>ab</sup> $\pm$ 0.21	0.40 <sup>ab</sup> $\pm$ 0.34	0.02 <sup>e</sup> $\pm$ 0.01
<b>0.77 kGy</b>	0.09 <sup>ab</sup> $\pm$ 0.06	0.21 <sup>bc</sup> $\pm$ 0.10	0.50 <sup>ab</sup> $\pm$ 0.20	0.05 <sup>cd</sup> $\pm$ 0.02
<b>1.13 kGy</b>	0.06 <sup>a</sup> $\pm$ 0.03	0.15 <sup>c</sup> $\pm$ 0.07	0.43 <sup>ab</sup> $\pm$ 0.37	0.05 <sup>abcd</sup> $\pm$ 0.02
<b>1.23 kGy</b>	0.14 <sup>bc</sup> $\pm$ 0.12	0.26 <sup>abc</sup> $\pm$ 0.13	0.62 <sup>b</sup> $\pm$ 0.51	0.05 <sup>abcd</sup> $\pm$ 0.02
<b>7 days of storage</b>				
<b>Non irradiated</b>	0.09 <sup>abc</sup> $\pm$ 0.05	0.21 <sup>bc</sup> $\pm$ 0.13	0.18 <sup>a</sup> $\pm$ 0.17	0.04 <sup>de</sup> $\pm$ 0.03
<b>0.77 kGy</b>	0.11 <sup>abc</sup> $\pm$ 0.04	0.19 <sup>bc</sup> $\pm$ 0.07	0.33 <sup>ab</sup> $\pm$ 0.28	0.04 <sup>de</sup> $\pm$ 0.02
<b>1.13 kGy</b>	0.15 <sup>c</sup> $\pm$ 0.10	0.24 <sup>abc</sup> $\pm$ 0.08	0.63 <sup>b</sup> $\pm$ 0.47	0.04 <sup>de</sup> $\pm$ 0.02

1.23 kGy      0.11<sup>abc</sup> ± 0.05      0.21<sup>bc</sup> ± 0.08      0.40<sup>ab</sup> ± 0.36      0.07<sup>ab</sup> ± 0.03

The results are reported as force in Newton (N). For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $P > 0.05$ ).

The Mexican blackberries (Table 16) do not show any tendency concerning the fracturability, adhesive force and rigidity. The firmness values indicated the fruit ripening over time.

**Table 16.** Texture parameters for non-irradiated and irradiated blackberry fruits from Mexico (fourth batch, second sampling). Average values ± SD for texture parameters of the samples in study (n=10).

	Fracturability ± SD	Firmness ± SD	Rigidity ± SD	Adhesive Force ± SD
<b>Immediately after irradiation</b>				
<b>Non irradiated</b>	0.09 <sup>abc</sup> ± 0.02	0.38 <sup>a</sup> ± 0.21	0.67 <sup>a</sup> ± 0.56	0.06 <sup>a</sup> ± 0.02
<b>1.10 kGy</b>	0.15 <sup>c</sup> ± 0.14	0.35 <sup>ab</sup> ± 0.15	0.44 <sup>abc</sup> ± 0.36	0.07 <sup>abc</sup> ± 0.03
<b>1.71 kGy</b>	0.13 <sup>bc</sup> ± 0.10	0.27 <sup>bcd</sup> ± 0.11	0.24 <sup>bcd</sup> ± 0.19	0.05 <sup>a</sup> ± 0.01
<b>2.64 kGy</b>	0.04 <sup>a</sup> ± 0.03	0.31 <sup>abcd</sup> ± 0.12	0.53 <sup>ab</sup> ± 0.55	0.07 <sup>abc</sup> ± 0.04
<b>3 days of storage</b>				
<b>Non irradiated</b>	0.09 <sup>abc</sup> ± 0.05	0.23 <sup>cd</sup> ± 0.10	0.13 <sup>cd</sup> ± 0.12	0.07 <sup>ab</sup> ± 0.06
<b>1.10 kGy</b>	0.14 <sup>bc</sup> ± 0.07	0.29 <sup>abcd</sup> ± 0.08	0.46 <sup>ab</sup> ± 0.39	0.09 <sup>bc</sup> ± 0.05
<b>1.71 kGy</b>	0.05 <sup>ab</sup> ± 0.03	0.33 <sup>abc</sup> ± 0.14	0.48 <sup>ab</sup> ± 0.23	0.10 <sup>c</sup> ± 0.05
<b>2.64 kGy</b>	0.08 <sup>abc</sup> ± 0.07	0.22 <sup>cd</sup> ± 0.09	0.31 <sup>bcd</sup> ± 0.30	0.07 <sup>abc</sup> ± 0.04

## 7 days of storage

<b>Non irradiated</b>	0.09 <sup>abc</sup> ± 0.08	0.27 <sup>abcd</sup> ± 0.12	0.37 <sup>abcd</sup> ± 0.25	0.07 <sup>ab</sup> ± 0.03
<b>1.10 kGy</b>	0.16 <sup>c</sup> ± 0.12	0.26 <sup>bcd</sup> ± 0.07	0.24 <sup>bcd</sup> ± 0.15	0.08 <sup>abc</sup> ± 0.03
<b>1.71 kGy</b>	0.05 <sup>ab</sup> ± 0.03	0.23 <sup>cd</sup> ± 0.10	0.07 <sup>d</sup> ± 0.08	0.08 <sup>abc</sup> ± 0.04
<b>2.64 kGy</b>	0.09 <sup>abc</sup> ± 0.05	0.21 <sup>d</sup> ± 0.04	0.34 <sup>abcd</sup> ± 0.14	0.05 <sup>a</sup> ± 0.01

The results are reported as force in Newton (N). For each parameter (columns) the values between treatments that have the same letters are not considered significantly different ( $P > 0.05$ ).

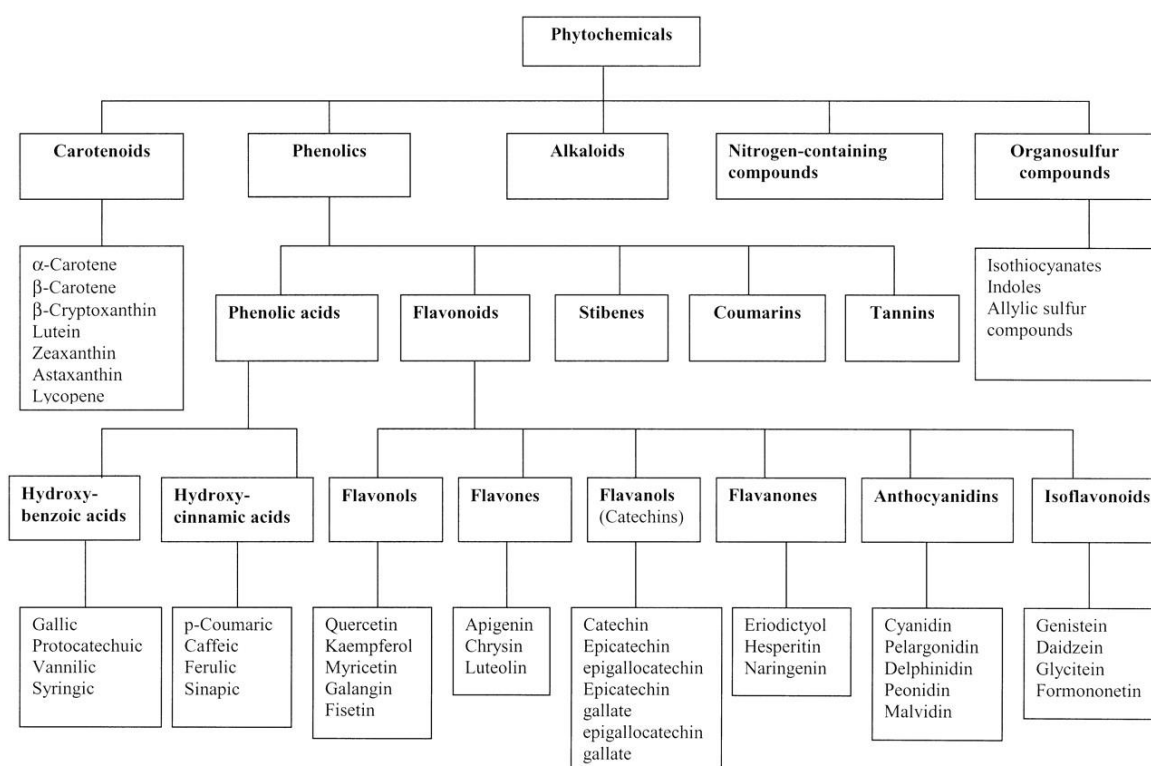
On apples, a decrease of firmness was observed immediately after irradiation with doses from 0.5 to 1.5 kGy. The firmness decrease was higher with higher doses (Al-Bachir, 1999). The same results were obtained for fresh strawberries irradiated with electron beam (e-beam) at 1 kGy and 2 kGy (Yu, 1996). A loss of firmness was also observed in apricots and peaches for irradiation doses higher than 0.6 kGy (Arvanitoyannis, 2010). Moreover, it was observed a decrease in texture in irradiated raspberries, however, the firmness of the raspberries treated with radiation doses of 1.0 and 1.5 kGy were not severely affected during the storage period (Cabo Verde, 2013).

*Chapter 5 - Chemical  
Characterization of  
blackberries*

## 5.1 Phytochemicals and health

### 5.1.1 Classification of phytochemicals

Among the most abundant phytochemicals are phenolics or polyphenols. These compounds are characterized by having one or more aromatic rings and one or more hydroxyl groups and are generally classified into phenolic acids, flavonoids, stilbenes, coumarins and tannins (Pereira, 2009). The Figure 28 show a schematic classification of phytochemicals.



**Figure 28.** Schematic classification of phytochemicals (adapted from: Os fitoquímicos e seus benefícios na saúde, 2009)

The most abundant polyphenols in blackberries are the flavonoids. Among these, anthocyanins are the group that is present in greater amount (the most significant is Cyanidin 3-O-glucoside with 138.72 mg/100g FW). Flavanols and flavonols are also

described as important constituents of blackberry (the most significant are Epicatechin with 11.48 mg/100g FW and Quercetin 3-O-galactoside with 4.10 mg/100g FW, respectively) (<http://www.phenol-explorer.eu/foods>).

### 5.1.2 Health effects

The interest in phytochemicals results from the evidence of several studies that reveal that a diet high in fruits and vegetables may decrease the risk of chronic diseases, such as cardiovascular disease and cancer. The phytochemicals including phenolics, flavonoids and carotenoids from fruits and vegetables may be a key in reducing chronic disease risk (Boyer, 2004).

Many polyphenols have been described as antioxidants. An antioxidant can be defined as any substance that even if present in low concentrations (as compared with the oxidizable substrate) has the ability to delay or inhibit oxidation of the substrate in an effective way (Shami, 2004). Antioxidants can have direct action in stopping action of free radicals or may be indirectly involved in enzyme systems with that function.

Many foods that are part of everyday diet are rich in antioxidants that promote the prevention and / or reduction of damage caused by free radicals in cells. In the plant kingdom many natural sources of antioxidants may be found. The antioxidant power is related to the composition of phenolic compounds of these foods products (Silva, 2011). As reported by numerous studies, the most important effects of polyphenols are anti-inflammatory, anticancer and neuroprotective properties, thus preventing cardiovascular and neurodegenerative diseases, diabetes and cancer (Seeram, 2008; Tavares, 2012; Paredes-Lopez, 2010).

Berries (*e.g.* raspberries, blackberries, blueberries, blackcurrants) contain micronutrients such as vitamin C and folic acid which are essential for health. However, berries may have additional health benefits as they are also rich in phytochemicals such as anthocyanins. In vitro studies indicate that anthocyanins and other polyphenols in berries have a range of potential properties. Such experimental data has led to numerous health claims on the internet implying that “berries are edible superstars that may protect against heart disease, cancers and ageing” (Beattie, 2005). Blackberry (*Rubus sp.*) fruit contains high levels of anthocyanins and other phenolic compounds,

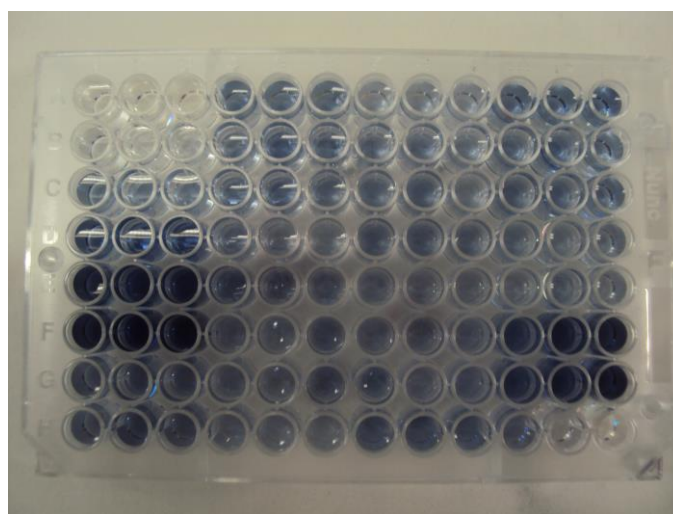
(mainly flavonols and ellagitannins) which contribute to its high antioxidant capacity and other biological activities. Blackberry phenolic compounds have protective effects on age-related neurodegenerative diseases and bone loss *in vivo* and can inhibit low-density lipoprotein and liposomal oxidation *in vitro*. Blackberry extracts have also exerted antimutagenic effects *in vitro* and *in vivo* by modifying cell signalling pathways and suppressing tumour promotion factors (Kaume, 2012).

### 5.1.3 *Methods for analysis of phytochemicals*

#### **FOLIN-CIICALTEU METHOD - DETERMINATION OF TOTAL PHENOLIC COMPOUNDS**

The method is based on the reduction of the phosphotungstic and phosphomolybdic acids present in the phenol reagent - Folin-Ciocalteu - by compounds existing in the sample, producing a blue colour which absorbs at 765 nm (Figure 29). The initial yellow colour turn to a blue colour after the reaction, allowing the quantification of the reduced substances. The reaction occurs in an alkaline medium which is obtained by the addition of sodium carbonate solution (2 M).

The Folin-Ciocalteu method is not specific, since it determines all naturally occurring phenolic compounds and reducing agents added, and they can therefore interfere with the results obtained (Silva, 2011).



**Figure 29.** Illustrative example of Folin-Ciocalteu method, producing a blue chromophore.

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography (HPLC) is an analytical technique that separates compounds from a sample, based on their different interactions between the mobile phase injected in the column and an appropriate stationary phase.

Compared to the traditional column chromatography, HPLC (Figure 30) is much faster since the mobile phase doesn't drip through the column under gravity but is forced through under high pressures of up to 400 atmospheres. Moreover, the stationary phase is made of particles with just a few micrometers and a high surface area available for the mixture components equilibrium between both phases. This increases the sensitivity and allows an improved separation analysis with very low volumes of injected samples. These features turn HPLC a very reliable technique with good performance, reproducibility and ease of result analysis (Naum, n.d.) also due to the variety of detection methods available, which are highly automated and extremely sensitive (<http://www.chemguide.co.uk/analysis/chromatography/hplc.html>).



**Figure 30.** Hitachi System LaChrom Elite® HPLC

The most commonly used HPLC method is the reversed-phase chromatography due to its reproducibility and broad applicability. The stationary phase is hydrophobic and consists of silica particles covalently bonded to alkyl chains. The octadecyl group (C18-bonded silica) is the most popular. The mobile phase is usually a mixture of water with a miscible, polar organic solvent (such as acetonitrile or methanol), which ensures a proper interaction of the sample compounds with the non-polar, hydrophobic particle surface of the stationary phase.

Compounds of an injected sample will flow through the column at different speeds provided they have (slightly) differences in polarity. Molecules more hydrophobic will be delayed because they are more strongly attracted to the particles of the stationary phase (van der Waals dispersion forces), whereas those more hydrophilic will be preferentially attracted to the mobile phase and are eluted first (<http://www.chemguide.co.uk/analysis/chromatography/hplc.html>).

Different compounds have different retention times (time between injection of sample until the display shows a maximum peak height for that compound). For a particular compound, the retention time will vary depending on: i) the pressure used (because that affects the flow rate of the solvent); ii) the nature of the stationary phase (not only what material it is made of, but also particle size); iii) the exact composition of the mobile phase; and iv) the temperature of the column (<http://www.chemguide.co.uk/analysis/chromatography/hplc.html>).

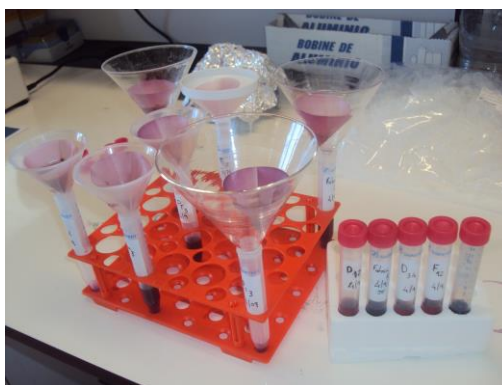
Many organic compounds absorb in the UV-Vis light range and may have various wavelengths of maximum absorption. Therefore, the use of an optical detector that measures the light absorbed by the mobile phase immediately after the HPLC column provides a valuable method to identify the separated compounds of an injected sample, by comparing the peaks' retention times with those of standards. Since the amount of light absorbed is proportional to the concentration of a particular compound (Beer-Lambert law), quantification is usually done by measuring the peak area (<http://www.chemguide.co.uk/analysis/chromatography/hplc.html>).

## 5.2 Methodology of chemical analysis

### 5.2.1 Extraction of polyphenols

For chemical analyzes were used twenty five grams of each sample irradiated and non-irradiated stored at -20 °C until extraction.

In the day of the extraction, the stored samples were ground and homogenized in a mill containing liquid nitrogen, to ensure that there were minimal losses (*e.g.* oxidation) and divided into three vials with 1g each (three extracts from each sample). To each vial was added 6 mL of an ethanol solution (H<sub>2</sub>O:EtOH (1:1)). Vials were incubated during 30 minutes in the dark and subsequently centrifuged on Eppendorf centrifuges at 3250 rpm for 10 minutes at room temperature. The samples were filtered with filter paper of 2 µm (Figure 31) and the tubes were closed under nitrogen and stored at -20 ° C.



**Figure 31.** Illustrative example of filtration of the samples after extraction procedure

### 5.2.2 Total Polyphenols methodology

The quantification of the phenolic compounds present in the samples under study was performed using the colorimetric method of Folin-Ciocalteu (Waterhouse, 2002), with Gallic acid as reference standard. After 30 minute incubation at 40°C, absorbance was measured at 765 nm in a PowerWaveXS spectrophotometer. The content of phenolic compounds present in the sample (Gallic Acid Equivalents - GAE) was determined by a calibration curve constructed with standard solutions of Gallic acid with different

concentrations (0, 50, 200, 400, 550, 700 mg/L). The concentration of phenolic compounds obtained from the calibration curve was applied a multiplying factor corresponding to the dilution that samples were subjected. Four readings for each of the three representative's extracts of each sample were made. The results in mg/L of GAE were converted into mg/g dry fruit.

### 5.2.3 Methodology of phytochemical profile

The extracts prepared were filtered again through 0.2 µm PVDF syringe filter to ensure the cleanliness of particles which might be aggregated in the column. The phytochemicals profiles were performed by Hitachi System LaChrom Elite® HPLC with EzCrom Elite *Software*, a model L-2130 gradient pump, a model L-2200 temperature-controlled auto sampler, a model L-2300 column ovens, and a model L-2455 diode array detection system (DAD). The mobile phase consisting of 100% (v/v) acetonitrile (solvent A) and 0.1% (v/v) formic acid in H<sub>2</sub>O (solvent B), used a flow rate of 0.7 mL/min and runs of 60 minutes. Each injected sample volume was 10 µl of each extract and separations were achieved in a Phenomenex Aqua C18 125 A (150 x 4.60mm x 3 µm), operated at 37°C. The gradient started with 100% B, reducing gradually until 60% B at 40 min. At 41 min changes to 0% B until 49 min and at 50 min changes to 100% B until the end of run. Three readings were made at 280, 320, 370 and 520 nm.

The most intense peak was selected for the evaluation of possible differences between the irradiated and non-irradiated blackberries for different times of analysis, for all batches (Annex III).

### 5.2.4 Statistical Analysis

The *STATISTICA* 9.1 program was used for the statistical treatment of the results values that have been tested by the significance of Wilks test in order to be able to use the ANOVA analysis. The post-hoc Fisher's LSD mean comparison test was used to evaluate the differences between the samples for a confidence interval of 95%.

## *5.3 Results and discussion of chemical analysis*

### *5.3.1 Total Polyphenols*

A total phenolics initial value of 1.63 and 1.97 mg/g of dry fruit was found for blackberries from the Netherland and Mexico (Table 17), respectively.

In blackberries from Netherland, a progressive increase of total polyphenols was observed for non-irradiated blackberries until seven days of storage (Table 17). The irradiated blackberries samples at 0.77 kGy showed the same behaviour that non-irradiated samples. However, blackberries irradiated at 1.23 kGy, exhibited significantly higher values than non-irradiated blackberries. This may suggest that phenolic compounds in blackberries may increase with irradiation dose and storage. A study on water of cork relates higher doses are able to destroy the benzene ring of the tannins and other phenolic. Without time rearrangement of the molecules there is an increase in the concentration of phenolic smaller structures (Madureira, 2011).

The non-irradiated blackberries from Mexico (Table 17) showed the highest total polyphenol value immediately after irradiation, a that decreased after three days of storage. For irradiated samples at 1.71 kGy and 2.64 kGy there was an increase in total polyphenols after three days of storage. Nevertheless, this trend has not maintained after seven days of storage. In fact it was observed a decrease in the content of phenolic compounds for all the irradiated samples.

**Table 17.** Phenolics (mg/LGAE) from non-irradiated and irradiated blackberry fruits from Netherland and Mexico (third and four batches, second sampling). Average values  $\pm$  SD for the samples in study (n=12; n=12).

<b>Phenolics totals (mg/g dry fruit) (Mean<math>\pm</math>DP)</b>			
<b>Blackberries from</b>	<b>Netherlands (batch 3)</b>		<b>Mexico (batch 4)</b>
<b>Immediately after irradiation</b>			
<b>Non irradiated</b>	1.63 <sup>abc</sup> $\pm$ 0.14	<b>Non irradiated</b>	1.97 <sup>f</sup> $\pm$ 0.18
<b>0.77 kGy</b>	1.30 <sup>a</sup> $\pm$ 0.04	<b>1.10 kGy</b>	1.04 <sup>a</sup> $\pm$ 0.02
<b>1.13 kGy</b>	1.78 <sup>bcdef</sup> $\pm$ 0.22	<b>1.71 kGy</b>	1.40 <sup>bcd</sup> $\pm$ 0.19
<b>1.23 kGy</b>	2.10 <sup>fg</sup> $\pm$ 0.25	<b>2.64 kGy</b>	1.36 <sup>bc</sup> $\pm$ 0.12
<b>3 days of storage</b>			
<b>Non irradiated</b>	1.68 <sup>bcd</sup> $\pm$ 0.15	<b>Non irradiated</b>	1.48 <sup>bcd</sup> $\pm$ 0.07
<b>0.77 kGy</b>	1.48 <sup>ab</sup> $\pm$ 0.15	<b>1.10 kGy</b>	1.55 <sup>cde</sup> $\pm$ 0.07
<b>1.13 kGy</b>	1.72 <sup>bcd</sup> $\pm$ 0.19	<b>1.71 kGy</b>	2.03 <sup>f</sup> $\pm$ 0.06
<b>1.23 kGy</b>	1.95 <sup>cdef</sup> $\pm$ 0.09	<b>2.64 kGy</b>	2.05 <sup>f</sup> $\pm$ 0.25
<b>7 days of storage</b>			
<b>Non irradiated</b>	1.98 <sup>cde</sup> $\pm$ 0.13	<b>Non irradiated</b>	1.59 <sup>de</sup> $\pm$ 0.13
<b>0.77 kGy</b>	2.05 <sup>ef</sup> $\pm$ 0.28	<b>1.10 kGy</b>	1.33 <sup>b</sup> $\pm$ 0.04
<b>1.13 kGy</b>	1.46 <sup>ab</sup> $\pm$ 0.18	<b>1.71 kGy</b>	1.70 <sup>e</sup> $\pm$ 0.12
<b>1.23 kGy</b>	2.39 <sup>g</sup> $\pm$ 0.35	<b>2.64 kGy</b>	1.52 <sup>bcd</sup> $\pm$ 0.02

The values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ).

A study of blackberries with three ripening reported that for total phenolic compounds, the values ranged from 5.8 to 5.2 mg GAE g<sup>-1</sup>, showing no specific trend (Acosta-Montoya, 2009). In oranges it was detected that phenolic compounds increased with irradiation dose and storage time (Moussaid, 2000). On the other hand  $\gamma$ -irradiated leaf extracts of *Nelumbo nucifera* did not show significant differences in total polyphenols (Jeong, 2009). Other authors referred that irradiation at 4 and 8 kJ m<sup>-2</sup> significantly increased total phenolic contents in tomato fruit (Chang-hong, 2012).

### 5.3.2 Phytochemicals profile

The absorption peak was measured at 520nm, a wavelength that characterizes the presence of anthocyanin's (Barreto, 2005). This peak was found to be present in all the analysed samples in the highest intensity. Table 18 show the values obtained for this peak in the different samples.

The results of Netherlands blackberries (Table 18) showed a gradual increase over storage time in absorbed intensity at this wavelength. This increase may reveal a fruit ripening. Blackberries irradiated with the higher dose showed the highest value at the different storage times studied.

For Mexican blackberries (Table 18) there was an increase of Phytochemical after three days of irradiation. For seven days of storage the non-irradiated and 2.64 kGy irradiated samples showed a decrease in intensity, the latter being more significant. These blackberries also show a very low value of phytochemicals, relative to others samples, immediately after irradiation.

**Table 18.** Phytochemical (mAU – peak area) of non-irradiated and irradiated blackberry fruits from Netherland and Mexico (third and four batches, second sampling). Average values  $\pm$  SD for the samples in study (n=9; n=9).

Phytochemical (mAU) (Mean $\pm$ DP)			
Blackberries from	Netherlands (batch 3)		Mexico (batch 4)
<b>Immediately after irradiation</b>			
<b>Non irradiated</b>	1.25x10 <sup>7a</sup> $\pm$ 2.62x10 <sup>6</sup>	<b>Non irradiated</b>	1.11x10 <sup>7a</sup> $\pm$ 1.33x10 <sup>5</sup>
<b>0.77 kGy</b>	1.15x10 <sup>7a</sup> $\pm$ 1.65x10 <sup>6</sup>	<b>1.10 kGy</b>	1.15x10 <sup>7a</sup> $\pm$ 2.07x10 <sup>5</sup>
<b>1.13 kGy</b>	1.47x10 <sup>7ab</sup> $\pm$ 1.76x10 <sup>6</sup>	<b>1.71 kGy</b>	1.62x10 <sup>7bc</sup> $\pm$ 2.22x10 <sup>6</sup>
<b>1.23 kGy</b>	1.26x10 <sup>7a</sup> $\pm$ 1.27x10 <sup>6</sup>	<b>2.64 kGy</b>	9.92x10 <sup>6a</sup> $\pm$ 1.07x10 <sup>6</sup>
<b>3 days of storage</b>			
<b>Non irradiated</b>	1.76x10 <sup>7abc</sup> $\pm$ 4.28x10 <sup>6</sup>	<b>Non irradiated</b>	1.98x10 <sup>7de</sup> $\pm$ 1.25x10 <sup>6</sup>
<b>0.77 kGy</b>	1.49x10 <sup>7ab</sup> $\pm$ 1.72x10 <sup>6</sup>	<b>1.10 kGy</b>	1.31x10 <sup>7ab</sup> $\pm$ 2.23x10 <sup>6</sup>
<b>1.13 kGy</b>	1.47x10 <sup>7ab</sup> $\pm$ 6.04x10 <sup>6</sup>	<b>1.71 kGy</b>	1.87x10 <sup>7cde</sup> $\pm$ 1.20x10 <sup>6</sup>

<b>1.23 kGy</b>	$2.24 \times 10^{7c} \pm 9.51 \times 10^6$	<b>2.64 kGy</b>	$2.07 \times 10^{7de} \pm 5.04 \times 10^6$
<b>7 days of storage</b>			
<b>Non irradiated</b>	$1.95 \times 10^{7bc} \pm 2.99 \times 10^6$	<b>Non irradiated</b>	$1.75 \times 10^{7cd} \pm 1.77 \times 10^6$
<b>0.77 kGy</b>	$2.09 \times 10^{7bc} \pm 9.22 \times 10^5$	<b>1.10 kGy</b>	$2.09 \times 10^{7e} \pm 9.22 \times 10^5$
<b>1.13 kGy</b>	$1.95 \times 10^{7bc} \pm 2.59 \times 10^6$	<b>1.71 kGy</b>	$1.98 \times 10^{7de} \pm 1.07 \times 10^6$
<b>1.23 kGy</b>	$2.18 \times 10^{7c} \pm 2.18 \times 10^6$	<b>2.64 kGy</b>	$1.22 \times 10^{7a} \pm 1.19 \times 10^6$

The values between treatments that have the same letters are not considered significantly different ( $p > 0.05$ ).

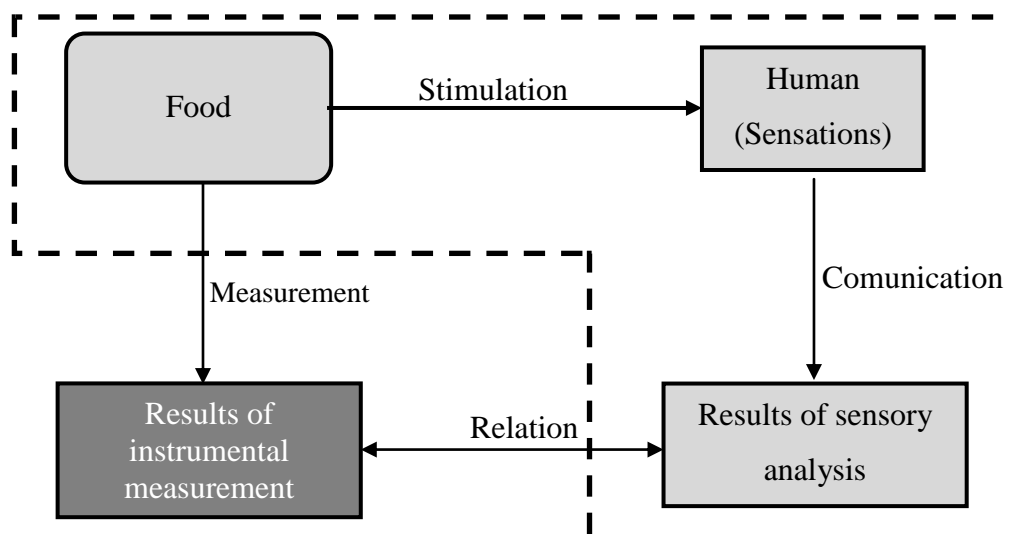
Anthocyanin content of blackberries is highly correlated with the colour of the fruit, and the higher the anthocyanin content the deeper the colour of the fruit is. Depending on the blackberry cultivar and maturity stage at harvest, as the fruit ripens, anthocyanin content may increase from 69.9 or 74.7 mg per 100 g of fresh fruit in unripe fruit to 164 or 317 mg per 100 g of fresh fruit in overripe fruit (Nunes, 2008). So, the lack of knowledge of the blackberries species in this study and its different origin from distinct parts of the world with totally different climate, habits and hours of transport, may have contributed to the different behaviour of the blackberries from Netherland and Mexico. A study of antioxidant capacity of tropical highland blackberry in three ripening stages related that major anthocyanin pigments increase from 0.20 to 1.34 mg.g-1 fresh weight. The same study refers that the tropical highland blackberry is a good source of antioxidants and contains appreciable levels of phenolic compounds, mainly ellagitannins and anthocyanins. (Acosta-Montoya, 2009).

*Chapter 6 - Sensory  
Analyses*

## 6.1 Sensory analysis

Sensory analysis is a scientific method used to measure, analyse and interpret the response a certain stimulus, through the achieved perception by one or more of the five senses (sight, touch, smell, taste and hearing). The stimulus can be translated into attributes (e.g. red colour or orange smell), on acceptance (e.g. like or dislike), in preference (e.g. I like this one better than that), among others.

According to EN ISO 5492:2009, the organoleptic quality is defined by the set of properties and characteristics of a product, giving it the ability to meet needs expressed or implied, being evaluated by consumers, consciously or unconsciously, through the sense organs.



**Figure 32.** Concept illustration of Sensory Analysis (adapted from Oliveira, 2011)

Sensory analysis (Figure 32) can be used for different purposes: i) evaluate the impact of reformulation or modification of product processing, ii) determine the validity iii) determine the profile of the product, iv) maintenance of quality standards; v) identify possible defects), and vi) evaluate preference and market studies (Lima, 2009).

## **SENSORY ANALYSIS LABORATORY**

Sensory analysis should be conducted in a sensory analysis laboratory, constructed and equipped in appropriate and standardized form for this purpose, according to Portuguese standard NP 4258:1993. The objective is to create an environment with minimal variations caused by external causes, in order to offer the fewest distractions to the taster. The tasting cabins should always be provided with sensory break materials (e.g. a glass of water and apple slices or crackers) (Lima, 2009; Louro and Nunes, 1988; Esteves, 2009).

### **TASTE PANEL**

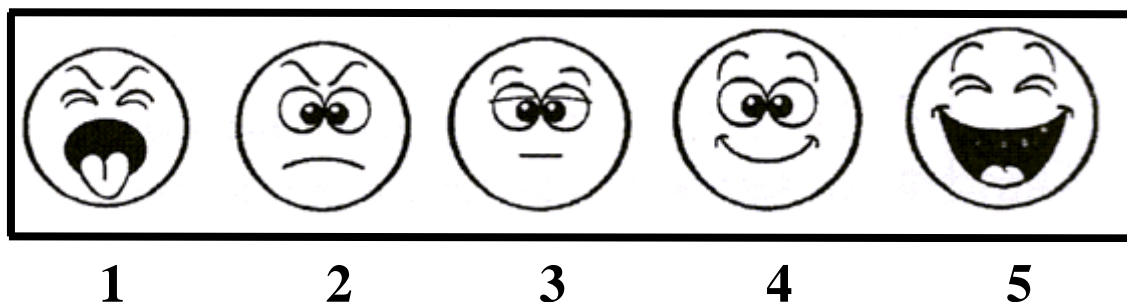
The panel of judges can be defined as being a group of individuals specially selected and trained to do, under certain conditions, a sensory evaluation with a known probability of error, eliminating the ratings at random. The panels are formed according to the purpose intended. There are two types of panels:

- i. Untrained panel – not demanding a tasting room, or trained panellists. The types of tests used are affective tests (preference, acceptance and ordering tests). Cannot be used as a measuring tool.
- ii. Trained panel - is selected based on the ability to see or distinguish stimuli. Requires a sensory analysis laboratory for tests and are regularly trained by varying the complexity of the tests - analytical tests (discriminatory and descriptive tests) (Lima, 2009).

### **ACCEPTANCE TESTS**

The acceptance tests are an intended proof with the purpose of inferring to what extent the consumer does not reject the product, through the expression of the degree of liking. The most common tests are proof of order and scale hedonic (Figure 33) (Lima, 2009; Louro e Nunes, 1988; Esteves, 2009).

Hedonic scale expresses like or dislike and was the test used in the study. The best are the scales with equal number of positive and negative categories.



**Figure 33.** Example of a hedonic scale (adapted from <http://smepirambu.blogspot.pt/2012/01/municipio-realiza-pesquisa-para-avaliar.html>)

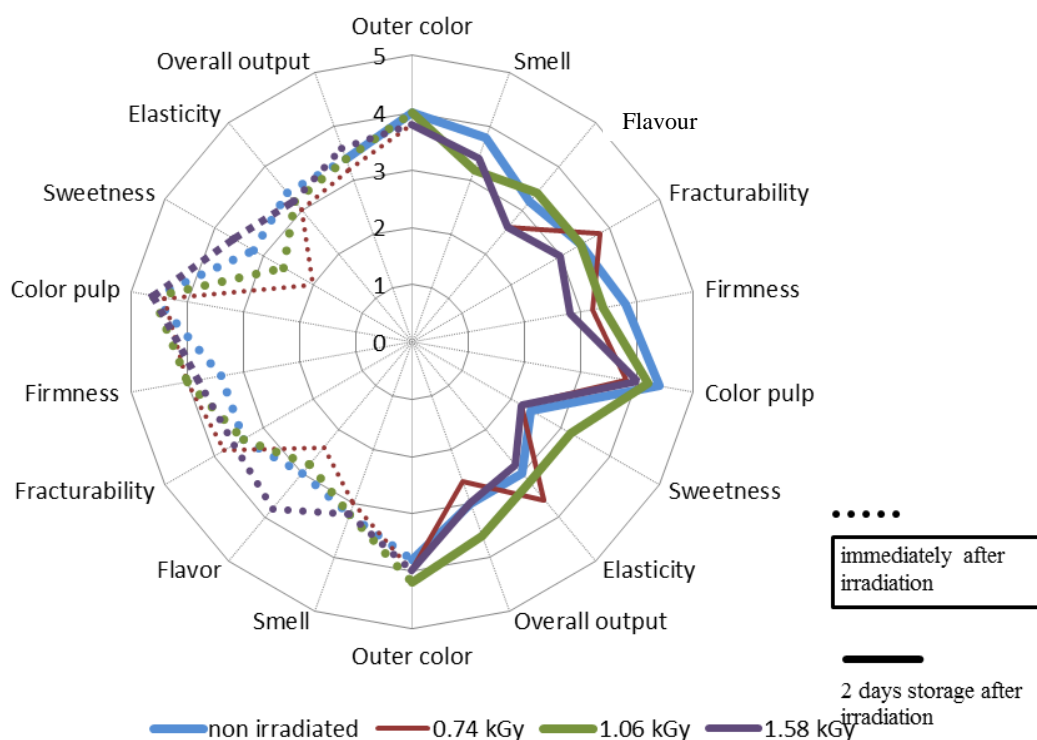
## 6.2 Methodology of the sensory analyses

Sensory tests of non-irradiated and irradiated blackberries at the different doses were performed primarily at CTN and the second sample at ITQB. For the first sample only the second batch was assessed, immediately after irradiation and with two days of storage. For the second sampling, sensory analyses were performed in the third and fourth batches, at the three tested storage times. Both samples were evaluated by an untrained panel consisting of 5 and 15 tasters, respectively. A test of acceptability of the product was carried out in order to assess to what extent the product could be refused/accepted by the consumer (Annex IV). An untrained test panel consisting of five randomly select individuals ( $25 < \text{age} < 50$ ; 40 % smokers, 100% healthy subjects) was performed to assess the sensory quality of samples and factors determining refusal or acceptability of the product by the consumer. Evaluated parameters were: 1) exterior and interior colour, 2) smell, 3) taste, 4) fracturability, 5) firmness, 6) sweetness, 7) elasticity and 8) overall assessment. For second sampling was added purchase intention of taster. A hedonic scale was used, ranging from 1 (dislike extremely) to 5 (like extremely).

The characterization of the sensory properties of blackberry fruits was analysed by the radar chart constructed with the ratings obtained for the different parameters evaluated by the panellists.

### 6.3 Sensory analyses results

Flavour and sweetness were the parameters where the evaluation was most different for blackberries from Portugal (Figure 34). Blackberries irradiated at 1.58 kGy showed the highest punctuation in both parameters immediately after irradiation. For this time, blackberries irradiated at 0.74 kGy show agreement with the values of TSS, being the less valued. However, after two days storage the samples rated as sweeter were the blackberries irradiated at 1.06 kGy, which is in agreement with TSS values. In evaluating the overall product the scores were concordant with the sweetness of blackberries, highlighting this parameter. Thus for both immediately after irradiation and two days of storage, the blackberries irradiated at 0.74 kGy were the least popular. The blackberries irradiated at 1.06 kGy and with two days of storage indicated a significant preference over the other doses, similarly to the sweetness parameter.



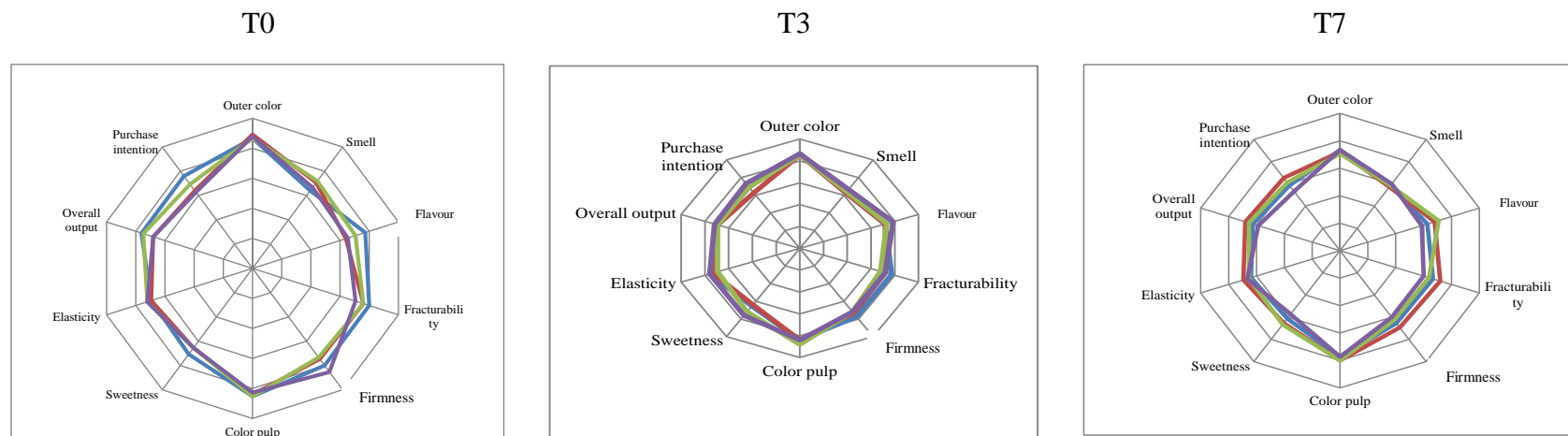
**Figure 34.** Comparison of various parameters studied in the sensory testing of blackberries irradiated at different doses and non-irradiated from the first sampling (blackberries from Portugal).

The non-irradiated blackberries from Netherlands (third batch), point out to be the most preferred in terms of flavour, sweetness and fracturability, revealing itself on the score of purchase intention and evaluation of the product in general (Figure 35), immediately after irradiation.

For three days of storage after irradiation there was a change in the scores of sweetness and flavour, indicating the non-irradiated and 0.77 kGy irradiated blackberry samples to be the less appreciated in these parameters. Conversely, the fruits irradiated at higher dose suggested to be the most appreciated in these parameters, showing consistent with the TSS assessments. The non-irradiated sample had the higher score for the fracturability in opposition to the obtained values by the texture analysis. After seven days of storage the blackberries irradiated at 0.77 kGy have the highest scores for fracturability and the irradiated at 1.23 kGy the lowest punctuation for this parameter, contrary to the verified in the blackberries texture assays. In accordance with the obtained TSS values, the blackberries irradiated at 1.13 kGy presented the highest values and the irradiated at 1.23 kGy the lowest of sweetness.

Regarding the sensory analysis of blackberries from Mexico (Figure 36) the irradiated samples at 2.64 kGy revealed the highest scores for both parameters sweetness and flavour until three days of storage. The non-irradiated sample after seven days of storage was evaluated with a high score for sweetness, which is in agreement with TSS results. This sample was the most punctuated for fracturability and hardness parameters along storage time that was consistent with the performed assessments of TSS and texture.

Variation of parameters of sensory analysis from blackberries from Netherlands (third batch)



Legend:

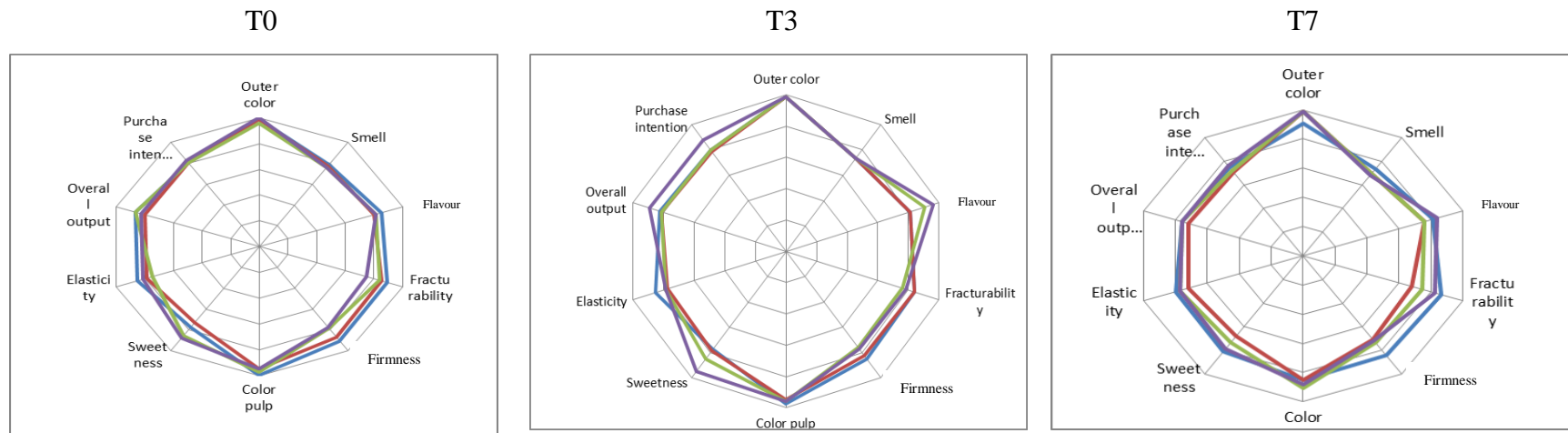
- non irradiated
- 0.77 kGy
- 1.13 kGy
- 1.23 kGy

1 - dislike extremely (middle)

5 - like extremely (outmost)

**Figure 35.** Comparison of variation of the parameters studied in sensory analysis of blackberries irradiated to different doses and non-irradiated blackberry fruit from Netherlands

Variation of parameters of sensory analysis from blackberries from Mexico (fourth batch)



Legend:

- non irradiated
- 0.10 kGy
- 1.71 kGy
- 2.64 kGy

1 - dislike extremely (middle)

5 - like extremely (outmost)

**Figure 36.** Comparison of the variation of the parameters studied in sensory analysis of the blackberries irradiated to different doses and non-irradiated blackberry fruit from Mexico

The flavour and sweetness seem to be the most important parameters for the tasters to set the punctuation of the product in general. For texture parameters the panellist showed a preference for the non-irradiated blackberries. However, overall, there are no significant differences in the evaluation of the tasters, indicating that the irradiated blackberries were accepted by the potential consumers.

In a study with minimally processed fruits and vegetables, it was observed that irradiation did not influence watermelon sweetness or pineapple sourness (Arvanitoyannis, 2009). In another study it was reported that blueberries exposed up to 1.6 kGy dose were found acceptable by the panellists in terms of overall quality, colour, texture, and aroma (Trigo, 2006).

*Chapter 7 - Conclusions  
and future work*

## 7.1 Conclusions

The first step was intended to assess methodologies in order to evaluate if there is any add-value by blackberries irradiation. Low microbial inactivation efficiency was verified for the applied gamma radiation doses in the preliminary study (first sampling – blackberries from Portugal). This study was important to redefine the methodological setup to assess the feasibility of irradiation as a conservation treatment for longer periods, therefore aiming to increase blackberry fruits shelf-life.

The effect of higher doses of irradiation (second sampling – blackberries from Netherland and Mexico) in the blackberries was investigated in an attempt to increase the reduction of the microbial population of blackberries; indeed an increased efficacy of microbial inactivation with the irradiation was observed. For the blackberries from Netherlands (batch 3) irradiated at 1.23 kGy an inactivation efficiencies above 90% was observed for total microbial population survival and 97% for fungal population.

Nevertheless, based on the obtained microbial inactivation results it was not verified an extension of shelf life of blackberries, after irradiation.

For the applied doses it was not observed an effect of gamma irradiation in the analysed properties with relevance to the consumer.

The colour parameters of non-irradiated blackberries from Netherlands presented the highest Hue value, compared to other blackberries, followed by blackberries from Portugal and after the blackberries from Mexico, immediately after irradiation, indicating different stages of maturation. Anthocyanin content of blackberries is highly correlated with the colour of the fruit, and can be seen that in the analysis of peak with the highest intensity, read at 520nm, the non-irradiated blackberries from Netherlands has higher values than the fruits from Mexico. Comparing the blackberries irradiated to 1.13 (batch 3) and 1.10 kGy (batch 4), immediately after irradiation, the previous behaviour is observed, as in the non-irradiated blackberries.

Concerning the evaluation of sweetness, the tasters seemed to agree with the obtained values of TSS. As an example, the blackberries from Portugal (batch 1 and 2) irradiated at 0.74 kGy have shown a lower TSS value immediately after irradiation and

consequently have less punctuation in sweetness parameter in the sensory test; however after two days of storage, the blackberries irradiated at 1.06 kGy have a highest value of TSS and by the tasters in that parameter. Another results obtained that relate these two tests can be observed in the other samples. The flavour and sweetness seem to be the most important parameters for the tasters to set the punctuation of the product in general.

For texture parameters, there was a decrease in the adhesive force and firmness with the storage time and increased irradiation dose. In fact, in texture parameters, the tasters showed a preference for non-irradiated blackberries.

Phytochemicals and polyphenols evaluations indicated some consistency. An evidence of this was obtained for the blackberries from Netherlands, during storage, where both values increased for non-irradiated samples. Another example is for the blackberries from México irradiated at 2.64kGy where the polyphenols and phytochemicals increased after three days of storage, reducing after seven days of storage.

Nevertheless, there was a different behaviour between certain parameters for the samples analysed. This may be due to the different origin of blackberries. The totally different climate conditions, blackberries cultivar and maturity stage at harvest, habits and hours of transport for exportation, are factors that cannot be discarded to contribute for the different behaviour that was observed. All parameters evaluated in this study, from microbiology until sensorial analysis are affected by internal and external conditions that in turn affect the fresh fruit. One example of this different behaviour is the Hue parameter obtained for blackberries from Netherlands that, decreased with irradiation and after seven days of storage, although the blackberries from Mexico have shown an opposite behaviour. For this reason there was no comparison, throughout the discussion of the results regarding inter-batches behaviour.

Summing up, it can be mentioned that an irradiation dose up to 2.64 kGy, does not result on a major impact on physical, chemical and physical attributes of blackberries. Sensory analysis showed acceptance for blackberries irradiation, according to panellists. Overall this work reveal that gamma irradiation treatment have high potential to be further evaluated as a conservation treatment for blackberry fruit.

## 7.2 *Future work*

Challenging tests with potentially pathogenic microorganisms on blackberries would be relevant to evaluate the efficiency of irradiation as a disinfection treatment. This will guarantee the food safety and further could allow the consumption of fresh fruit by risk group population.

Despite the current knowledge of their chemistry, research specific to blackberry phenolic compounds' health benefits, metabolism, bioavailability, and mechanism by which they confer health benefits is scarce (Kaume, 2012).

The antiobesity, antidiabetic, antimicrobial, and anti-inflammatory properties of blackberry phenolic compounds need investigation. Similarly, studies that elucidate the *in vivo* physiologically effective concentrations of blackberry phenolic compounds are necessary. A large number of samples and studies are necessary to provide a better understanding of effects of ionizing radiation on chemical and nutritional structure of blackberries, obtaining more conclusive results and possibly spanning further analysis (*e.g.* spectrum of sugar, antioxidant activity and ascorbic acid).

Based on results, higher doses should be tested in order to conclude whether it is possible to extend the shelf life of blackberries (important for export), without causing changes in properties of relevance to the consumer.

To complete an economic study of the industrial process of blackberries using ionizing radiation as treatment should be carried out to determine if this technology is a good alternative in all enveloping aspects on manufacturing process and later consumption.

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*Annex I -  
Validation of the method  
for determining the  
microbial load  
of blackberries for treatment  
with ionizing radiation*

## 1. Objective and product characterization

The objective of this study is the identification and quantification of microbial species existing blackberries and verification of the efficacy of treatment with ionizing radiation to different dose rates. A wide variety of fungi (mostly moulds) is capable of growing and spoiling various types of berries; considering the fact that these commodities contain high levels of sugar and other nutrients and a water activity, ideal for fungal growth. Additionally, the low pH of these fruits eliminates the competition from many bacterial species, making it easier for fungi to grow and spoil the fruits [8].

The blackberries are subjected to a treatment with ionizing radiation in order to inactivate microbial contaminants. Will be evaluated the contamination of microorganisms in each dose rate.

## 2. Methodology

It is necessary to make up an identification and quantification of microorganisms - (CFU/mL) in the sample. To do so, it proceeds to spreading techniques. The TSA it is a culture medium selected for the evaluation of total microbiota population present in the samples. This medium is typically used for growth in general. As described in numerous studies the fruit contains mostly fungi in microbial contamination. Indeed to verify the impact of irradiation doses studied in this microorganism, we used MEA medium. This is above all indicated for growth in fungi and yeast. Subsequently the samples were subjected to treatment with ionizing radiation using 125g of transport boxes to prevent further handling. Tests will be carried out with doses of 0.5, 1, 1.5 and 2 kGy (3 doses per batch).

After this treatment it is the microbial resistant to treatment and compared with untreated sample and subjected to various doses.

## 3. Sampling

The blackberries are collected and transported from by the producing companies. The samples will be transported to the microbiology laboratory of ITN - Nuclear and Technological Institute and maintained at refrigeration temperatures from collection until analysis. The analyses shall be performed as soon as possible.

## 4. Experimental procedure

For each sample were performed two or three repetitions (depending on the total amount of sample for analysis) for dose to assess the reproducibility of the process. Each sample blackberries for microbiology contained  $\approx 25$ g of fruit.

All procedures are performed using aseptic techniques and a laminar flow.

### Material and equipment:

- Chamber of horizontal laminar flow
- Bunsen Beak
- Sacks of stomacker
- Stomacker
- Physiological serum + 0.1% Tween80
- Petri plates of Tryptic Soy Agar (TSA) and Malt Extract Agar (MEA)
- Sterile Test tubes
- Sterile pipettes 0.1, 1 and 50 mL
- Pipettor
- Sterile spreaders
- Greenhouse

### **i. Preparation of the samples after irradiation**

After irradiation the samples were placed in a sterile sack stomacker which was added 100 ml of physiological saline + 0.1% Tween 80 for better removal of microorganisms. The sacks were placed in stomacker for 15 minutes for better mixing.

## **ii. Validation of the method for determining microbial load**

To understand which sample quantities needed for a better assessment (between 30 to 300 CFU/plate) of the initial microbial load of blackberries, will carry the scattering of 0.1 and 1mL.

1. Identify the plaques (sample date, medium, sample volume, No. replica, state of the sample (not irradiated, irradiated each dose))

**For all samples, the procedure was performed in the same way.**

2. Mix the solutionSe necessária a diluição:
3. If necessary proceed to dilution of sample: placement of 1 mL of sample in sterile tube with 9 ml of physiological saline and shaken on a vortex mixer (make further dilutions from here if necessary).
4. Remove 0.1 mL sample directly and put on the plate. The same is done for sample amounts above this value.

### **Description of the technique: Direct Spread**

- a) Dispense 0.1 mL of the sample with the medium TSA plates.
  - b) Spreading movement is done in circulating using sterile spreader the L-shaped some grip to be felt.
  - c) Perform the same procedure to 1mL sample.
5. It will be three replicates of scattering direct for each 0.1 and 1mL spreading.
  6. Incubate the plates inverted at 32 ° C and count of colonies at 24, 48 and 72, 5 and 7 days.

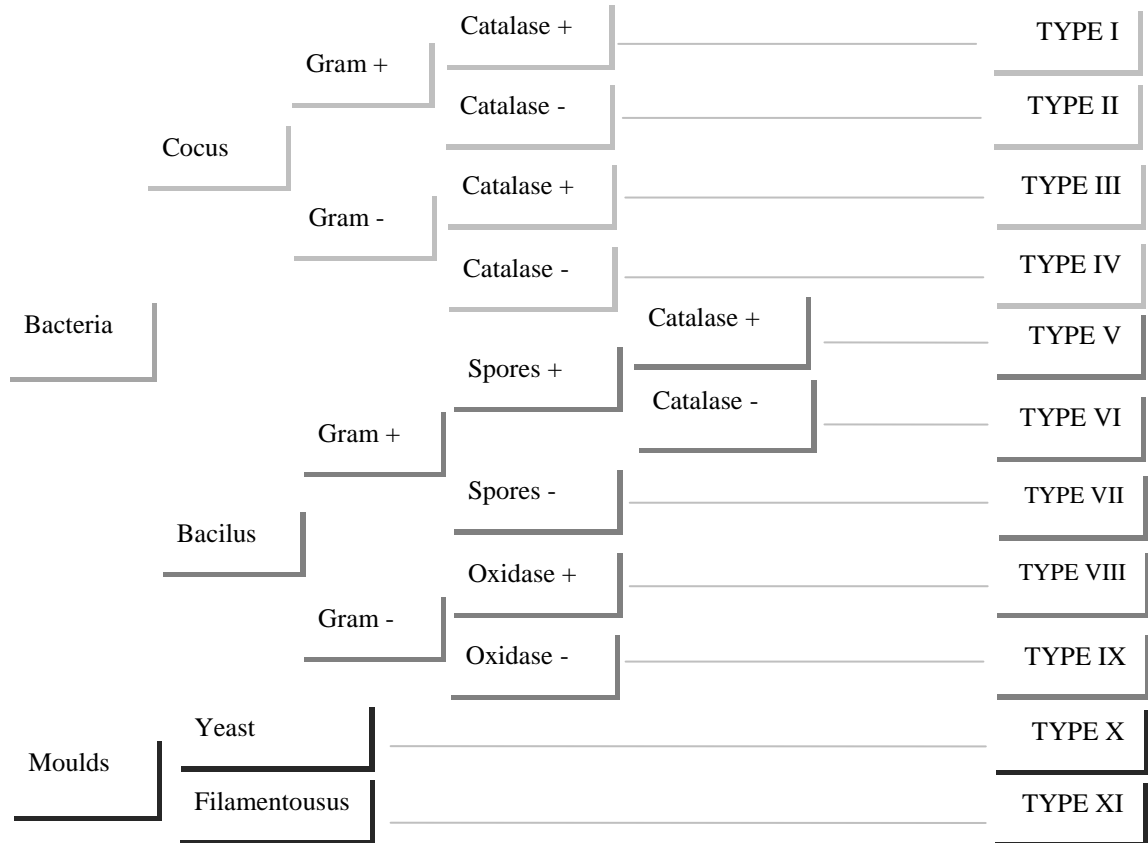
For the MEA the procedure is the same, but the incubation is performed at 28 ° C.

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*Annex II -  
Microorganisms typifying  
scheme*

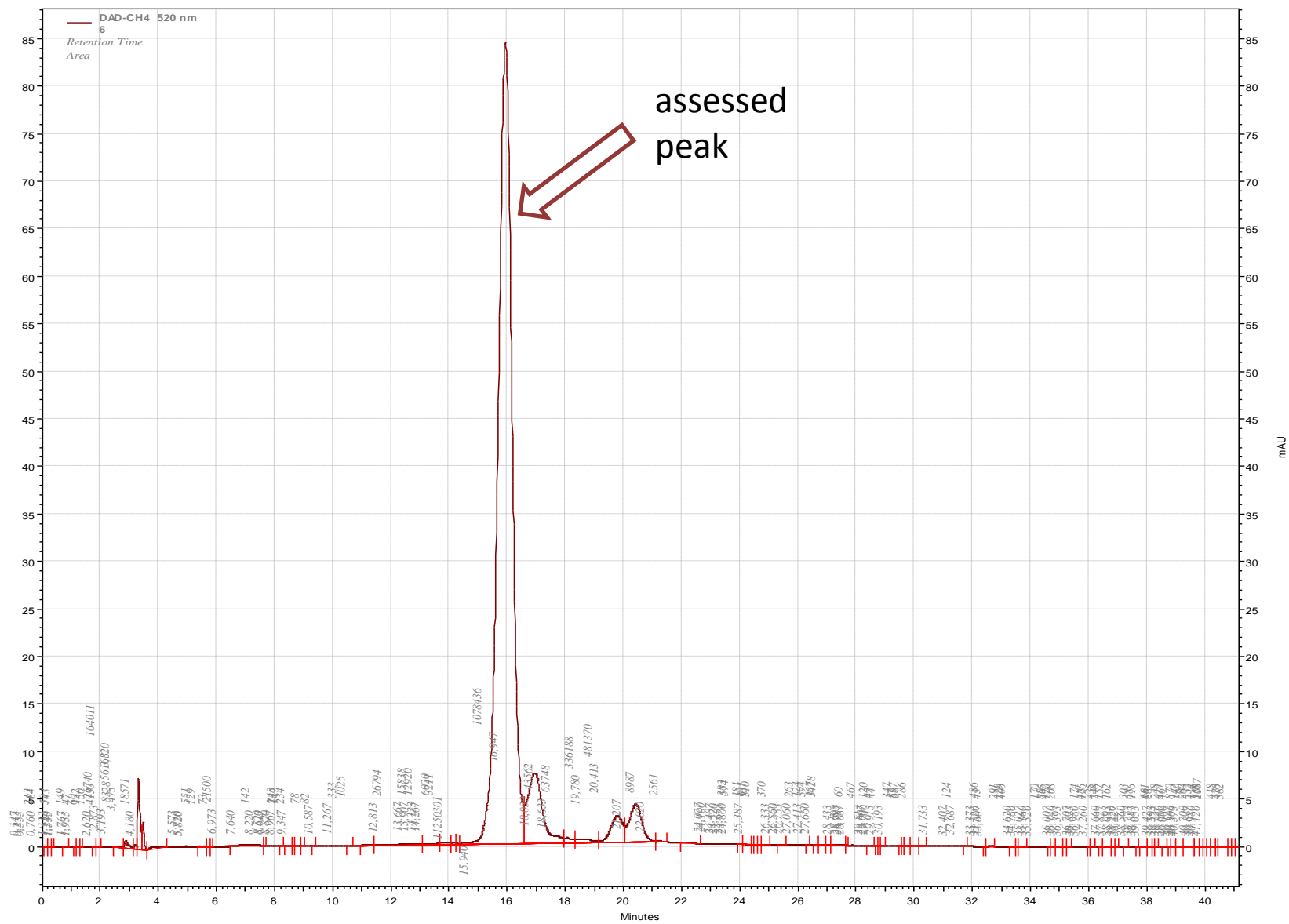
**Typifying of Microorganisms**



Adapted from: 1st edition of "Bergey's Manual of Systematic Bacteriology"

*Annex III - Example*  
*of the figures obtained by*  
**HPLC at 520 nm**

Annex III - Example of the figures obtained by HPLC at 520 nm



*Annex IV -*  
*Acceptability test*  
*(sensory)*

### Acceptability test

Name \_\_\_\_\_

Date:    /    /   

Evaluate each of the coded samples and use the scale below to indicate how much liked or disliked:

- 1 - Very unpleasant
- 2 - Unpleasant
- 3 - Indifferent
- 4 - Pleasant
- 5 - Very Pleasant

#### Outer colour

	1	2	3	4	5
174					
495					
523					
889					

#### Smell

	1	2	3	4	5
174					
495					
523					
889					

#### Flavour

	1	2	3	4	5
174					
495					
523					
889					

#### Fracturability of fruit skin

	1	2	3	4	5
174					
495					
523					
889					

#### Firmness (hardness)

	1	2	3	4	5
174					
495					
523					
889					

#### Colour pulp

	1	2	3	4	5
174					
495					
523					
889					

*Annex IV - Acceptability test*

**Swetness**

	1	2	3	4	5
<b>174</b>					
<b>495</b>					
<b>523</b>					
<b>889</b>					

**Elasticity (formation of "gum" in the mouth)**

	1	2	3	4	5
<b>174</b>					
<b>495</b>					
<b>523</b>					
<b>889</b>					

**Overall output**

	1	2	3	4	5
<b>174</b>					
<b>495</b>					
<b>523</b>					
<b>889</b>					

**Purchase intention**

	1	2	3	4	5
<b>174</b>					
<b>495</b>					
<b>523</b>					
<b>889</b>					

Comments: \_\_\_\_\_  
\_\_\_\_\_

Note: The parameter of purchase intention was only used in second sampling.