

## POSTHARVEST EVOLUTION OF PECTINOLYTIC ERWINIA AND ERWINIA SOFT ROT DURING COLD STORAGE OF CONSUMPTION POTATOES PRODUCED IN ALGERIA

Ammar Tiaiba<sup>\*1,2,3</sup>, Boubekour Seddik Bendahmane<sup>3</sup>

### Address(es):

<sup>1</sup> Higher School of Agronomy, ex-Hall of Technology, Kharouba, 27060, Mostaganem, Algeria.

<sup>2</sup> University of M'sila, Department of Agricultural Sciences, BP 166, 28000, M'sila, Algeria.

<sup>3</sup> Plant Protection Laboratory, University of Mostaganem, RN 11, 27000, Mostaganem, Algeria.

\*Corresponding author: [ammartiaiba@univ-msila.dz](mailto:ammartiaiba@univ-msila.dz)

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### ABSTRACT

In Algeria, potatoes ranks second among the twenty main food crops. It's characterized by periods of overproduction corresponding to seasonal crops. Except these periods, supply tends to fall. In order for the availability of potatoes to be constant throughout the non-harvest periods, it is imperative to store the surplus production to ensure a constant supply during periods of unavailability of a new harvest. The storage of the potato must maintain a satisfactory quality and minimize losses and the postharvest development of bacteria and fungi. This work aims to identify and follow the evolution of pectinolytic *Erwinia* and *Erwinia* soft rot during storage at 4 and 10° C of two varieties of potato grown in Algeria, i.e. Spunta and Désirée. The results indicated the presence of three species of *Erwinia*: *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*. Initially, for both varieties, *E. carotovora* subsp. *carotovora* predominates followed by *E. carotovora* subsp. *atroseptica* and finally *E. chrysanthemi*. During storage, the three species evolve almost in the same way and their multiplication is more important at 10° C than at 4° C, particularly on the Spunta. About the total bacteria, the highest levels are recorded on Spunta, especially at 10° C. In addition, the *Erwinia* spp. / total bacteria ratio showed that *Erwinia* spp. are very most abundant compared to the total bacteria. The assessment of soft rot losses has also shown that they are greater in the Spunta variety whether at 4 or 10° C.

**Keywords:** *Erwinia* spp., Postharvest soft rot, Potato, Variety, Storage temperature

### INTRODUCTION

As a result of agricultural policy and increased demand, the potato is consolidating its place in the economy, agriculture and eating habits of the Algerian people. In fact, it ranks second among the twenty main food crops. In near future there will be an evolution in demand for potatoes and potato-based products as a result of changes in lifestyles and food habits. This will inevitably lead to a multiplication of processing units using this product, which requires stability of supplies over time. In Algeria, the potato production is often characterised by periods of overproduction corresponding in particular to seasonal crops. Except during this period, supply tends to fall and prices are increasing. In order for the availability of potatoes to be as constant as possible throughout the non-harvest periods and to avoid large price fluctuations, it is necessary to resort to the storage of surplus production to ensure a constant supply during periods of unavailability of a new harvest. However, both for fresh consumption and for industrial processing, the storage of the potato must maintain a satisfactory quality of the product and minimize losses due to sprouting and desiccation as much as possible, but also those due to rotting and the postharvest development of pathogen like bacteria and fungi.

Variety, temperature, relative humidity and storage time are the main factors in maintaining the quality of stored potato tubers. In practice, the choice of storage temperature is a compromise between a relatively high temperature (8-10°C) in order to avoid a low temperature sweetening and a lower temperature ( $\leq 5^\circ$ ) to limit weight loss, rotting loss, sprouting and senescence sweetening (Gravouille et Poupard-Caron, 1997). In Algeria and for short storage times, the professionals store their potatoes around 10° C, unlike in the case where the storage times are long, the tubers are often stored at 4° C.

During storage, potato tubers may exhibit various rots that affect commercial quality and, in some cases, cause significant loss in stocks (Cools et al., 2014). The majority of authors identify two main types of rots. Fungal dry rots caused mainly by *Fusarium* spp. (Tivoli, 1996; Singh et Sharma, 2018) and soft rots usually caused by bacteria (Priou et Jouan, 1996), the typical example of which remains that due to pectinolytic *Erwinia* (Hyman et al., 2001; Hua et al., 2020). Although they can appear in vegetation, it is during storage that *Erwinia* species and *Erwinia* soft rots cause the most damage and loss (Mills et al., 2006).

Three species of *Erwinia* are responsible for blackleg and soft rot in potatoes, *Erwinia carotovora* subsp. *atroseptica* [(Van Hall 1902) Dye 1969], *Erwinia*

*carotovora* subsp. *carotovora* [(Jones 1901) Bergey, Harrison, Breed, Hammer and Huntton, 1923] and *Erwinia chrysanthemi* [Burkholder, McFadden and Dimock 1953] (Perombelon et Van Der Wolf, 1998; Helias, 1999; Smadja et al., 2004; Singh et Sharma, 2018; Dourado et al., 2019). The geographic distribution of the three species is often determined by their thermal needs (Priou et Jouan, 1996; Afek et Orenstein, 2001; Horváth et al., 2002; Smadja et al., 2004).

*Erwinia carotovora* subsp. *atroseptica* is generally associated with the symptoms of potato blackleg; a disease which occurs during vegetation, particularly in temperate regions, its thermal optimum is between 15 and 20° C (Helias, 1999). *Erwinia carotovora* subsp. *carotovora* extends over very large geographic areas and a wider host range, and is responsible for the postharvest soft rot of many fruits and vegetables including potatoes (Reverchon et al., 2016; Li et al., 2020). Moreover, it seems have better conservation capacities beyond its hosts (in soils and water). It adapts to temperatures ranging from 20 to 37-40° C (Perombelon et al., 1987; Yap et al., 2004). *Erwinia chrysanthemi* is a common bacterium in tropical and sub-tropical environments. It grows at temperatures ranging from 25 to 37-40° C (Priou et Jouan, 1996).

During storage, once established, soft rot caused by *Erwinia* spp. leads to rapid contamination of the tubers. The action of exocellular bacterial enzymes, especially pectinolytic enzymes, cause the liquefaction and collapse of cell walls (Laurent et al., 2001; Kang et al., 2016), in contact with air, the cell content turns brown following the oxidation of phenols and gives off an odour characteristic foul-smelling. *Erwinia* soft rot is favoured by a confined atmosphere, excess humidity and wounds that facilitate the penetration of bacteria usually present on the surface or lodged in the lenticels of tubers. The severity of soft rots still depends on the genotype, physiological age, healing ability, and the calcium and water content of the tubers (Yap et al., 2004; Wu et Rioux, 2010).

This work was carried out with the objective of studying the evolution of pectinolytic *Erwinia* and postharvest *Erwinia* soft rot during cold storage of potatoes belonging to the Spunta and Désirée, which are extensively produced and consumed in Algeria. The study also focuses on the development and evolution of *Erwinia* spp. and the rot losses of potatoes during storage at 4 and 10° C; temperatures frequently practiced in the storage of potatoes intended for consumption and industrial processing in Algeria.

**MATERIAL AND METHODS**

**Plant material**

Two potato varieties were selected for the study. Desirée (red skin variety) and Spunta (yellow skin variety). These varieties take their origin from Ain Defla (Algeria), a region where potato is most cultivated. The harvest was carried out at maturity (seasonal crop), and according to the usual practices. The tubers have been carefully sorted to maintain only those with a medium and homogeneous size. Injured, suspect tubers and tubers with necrosis are also eliminated. The packaging was carried out in commercial PVC (Poly Vinyl Chloride) boxes.

**Experimental design**

The study consists of refrigerating potato tubers at 4 and 10° C; temperatures often practiced by Algerian professionals in the storage of potatoes. The potato tubers were allocated into four lots of four boxes each, with 25kg of potatoes per box considering two classification criteria: storage temperature with two levels (4 and 10° C) and variety with two modalities (Desirée and Spunta). The experimental lots are identified in Table 1.

**Table 1** Identification of experimental lots

Variety	Storage temperature	
	04° C	10° C
Desirée	D4 (lot of the Desirée, stored at 04° C)	D10 (lot of the Desirée, stored at 10° C)
Spunta	S4 (lot of the Spunta, stored at 04° C)	S10 (lot of the Spunta, stored at 10° C)

**Storage of experimental lots**

The storage of the experimental lots was carried out in the dark, in two cold rooms placed at our disposal by MAG-MOS company ex-ENAFROID, located in Mostaganem (Algeria). The temperature in the two rooms was 4±1° C and 10±1° C respectively. Throughout storage, the relative humidity (RH %) in the two rooms was between 75 and 78%.

**Erwinia soft rot losses**

The assessment of rot losses was limited to those due to Erwinia soft rot. For this purpose the soft rot rate was estimated by relating the number of tubers exhibiting soft rot to the total and initial number of tubers per box according to the formula below. Rotten tubers were removed from the boxes at each assessment and used for the diagnosis of alterations.

$$\text{Soft Rot Rate (\%)} = \frac{\text{Number of tubers with soft rot}}{\text{Total number of tubers}} \times 100$$

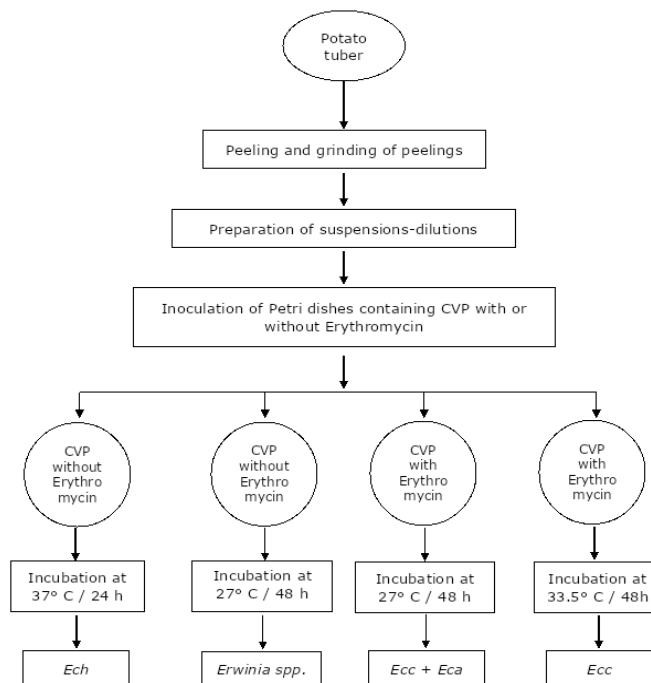
**Identification and enumeration of pectinolytic Erwinia**

*Erwinia carotovora* subsp. *atroseptica* (*Eca*), *E. carotovora* subsp. *carotovora* (*Ecc*) and *E. chrysanthemi* (*Ech*) form typical depressions or cavities on the modified Crystal Violet-Pectate (CVP); selective diagnostic medium (Tab. 2), and can be identified and quantified as a function of these cavities (Perombelon et al., 1987; Arias et al., 1998; Perombelon et Van Der Wolf 1998; Hyman et al., 2001). This medium is supplemented with 0.5g of tryptone to improve the growth of *Erwinia* spp. and the formation of cavities (Perombelon et Burnett 1991). The identification and enumeration of *Erwinia* species were performed according to a method developed by Perombelon et al. (1987). This method exploits the differential effect of three incubation temperatures (27, 33.5 and 37° C) and the presence or absence of Erythromycin (antibiotic) in the modified CVP medium on the formation of cavities. *Eca* forms cavities only at 27° C in the presence or not of Erythromycin, *Ecc* at 27 and 33.5° C but not at 37° C with or without Erythromycin and *Ech* at all temperatures but only without the Erythromycin. *Erwinia* spp. forms cavities only at 27° C without the presence of Erythromycin.

**Table 2** Composition of the modified CVP medium, (Perombelon et al., 1987)

Component	Amount
Aqueous crystal violet (0,075 %)	02 ml
NaOH (1N)	09 ml
CaCl <sub>2</sub> , 2H <sub>2</sub> O (10 %)	12 ml
NaNO <sub>3</sub>	02 g
Trisodium citrate	05 g
Sodium lauryl sulphate (10 %)	01 ml
Actidione (2%)	05 ml
Streptomycin	100 µg/ml
Sodium polypectate	18 g
Agar-agar	04 g
Water q.s.	1000 ml

Two medium-sized tubers, taken at random from each of the four boxes of the different experimental lots, are well washed with sterile water and then peeled so as to remove in addition to the skin 3 to 4mm approximately from the cortex, because according to Perombelon et Van Der Wolf (1998) and Hyman et al. (2001), the *Erwinia* species responsible for tuber soft rot is located at this level. The peels are then finely ground in a laboratory vegetable grinder (Retsch® Grindomix GM200, Mettmann, Germany). 1g of the ground peelings is added to 9ml of sterile physiological water (8.5g of NaCl in 1liter of distilled and sterile water) (Guiraud et al., 2012). Successive decimal dilutions are then prepared. The Petri dishes containing the modified CVP medium, without or with 35µg.ml<sup>-1</sup> of Erythromycin, are then inoculated, each with 0.1ml of the suspension (Fig. 1).



**Figure 1** Identification and enumeration diagram of pectinolytic Erwinia.

**Total bacteria**

The enumeration of the bacterial flora was performed using a stock suspension, which constitutes the base solution for the preparation of the suspension-dilutions. The stock suspension was prepared by dispersing 1g of finely ground peelings in 9ml of sterile physiological water. The ground peel was obtained using the same methodology as that applied for pectinolytic Erwinia. The non-selective medium used to be a nutrient agar known as PCA (Plate Count Agar), which is composed of (for one liter): 5g peptone, 2.5g yeast extract, 1g glucose and 15g agar-agar (Guiraud et al., 2012). This basic culture medium is supplemented with Actidione (Cycloheximide) at 0.1g/liter after sterilization. Actidione is an antifungal agent primarily against yeasts which may interfere with the analysis by their development on Petri dishes. The inoculated dishes are incubated at 30° C., the enumeration is carried out 72 hours after (Richard-Molard et Cahancier, 1984; Larrigaudiere et al., 1987; Guiraud et al., 2012). Each time when total bacteria and *Erwinia* spp. are counted and in order to estimate the importance of pectinolytic Erwinia in the total bacterial flora of stored tubers, the percentage of *Erwinia* spp. is then calculated according to the following formula.

$$\text{Erwinia spp. percentage (\%)} = \frac{\text{Number of Erwinia spp.}}{\text{Number of total bacteria}} \times 100$$

**Data analysis**

Results for all measured parameters were analysed using a three-factor experimental design. The studied treatments are variety, temperature and storage time. ANOVA tests were performed at the 0.05 and 0.01 levels, the treatment means were also compared using the Newman and Keuls test at the 5% level. SAS software (Statistical Analysis System, SAS System 9.4, SAS Institute Inc., USA) was used in this analysis.

**RESULTS AND DISCUSSION**

It has already been pointed out in methodology that during the rots checks we proceeded to eliminate the rotten tubers, because it is obvious that the development of rots without sorting would have been otherwise. However, at both storage

temperatures and for both varieties, *Erwinia* soft rot set in at almost the same time and continue to increase with increasing storage time (Fig. 2). From the 12<sup>th</sup> week of storage, the differences between the various levels of rotting amplify as the storage period lengthens. For this purpose, storage temperature and variety had significant effects ( $p < 0.01$ ) and differences in rot levels were very important. At the end of the 21<sup>st</sup> week of storage, the soft rot losses in the variety Spunta are higher than those in the Désirée. However, at 4° C these losses were around 1.7% for the Spunta versus 1% for the Désirée, while at 10° C, they were 3 and 2.5% respectively.

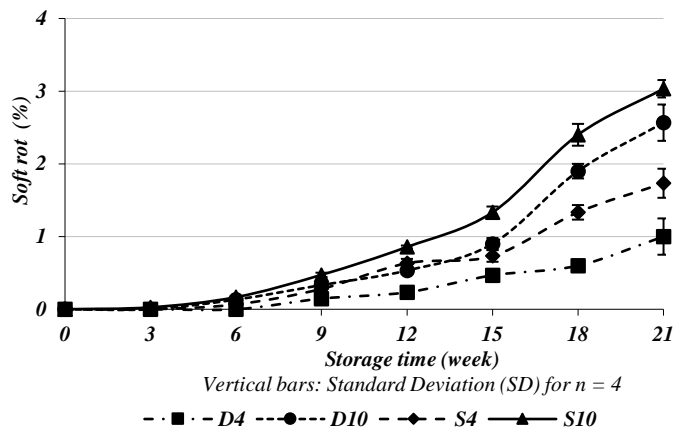


Figure 2 Evolution of *Erwinia* soft rot during storage.

For both varieties stored at the same temperature, soft rot sets in simultaneously. This is probably due to the same cultural origin of the tubers. According to Tsai et al. (2006), Afek et Orenstein (2001) and Laurila (2004), potato rots originate in the phase of cultivation where contamination generally begins. Jobling (2000), Martin (2004) and Mills et al. (2006) report other factors involved in the development of postharvest rots, like initial inoculum, handling and mechanical damage and the speed of healing and suberization, which is a varietal characteristic.

During storage, it is the relative humidity, the storage temperature and the sensitivity or resistance of the variety to pathogens and microbial growth that determines rotting losses. Khan et Wahid (1978), in a study on the Désirée and Ultimus potato varieties, argue these three factors as the main reasons for the differences recorded between the rates of rot losses during storage. They report that a lot of the Ultimus variety would be completely destroyed in the 10<sup>th</sup> week of storage at a temperature ranging between 25 and 35° C and a relative humidity of 40-50%, while at this same period, the rot rates for the Désirée variety only reached 20%. At 15° C and 60 to 70% relative humidity, rotting losses are 12 and 7% respectively.

Bacterial flora, which is initially more or less comparable for the two varieties, undergo significantly different evolutions during storage ( $p < 0.01$ ) depending on the variety and the storage temperature. During the first nine weeks, there was a strong bacterial proliferation, particularly on the tubers of the Spunta variety stored in 10° C. Beyond this period, bacteria observes a slight drop in levels to resume multiplication again during the last six weeks of storage (Fig. 3). Overall, the highest levels were recorded in Spunta lots, both at 4 and 10° C. However, bacterial contamination on Désirée variety remained low and exceeded slightly than on Spunta towards the end of storage.

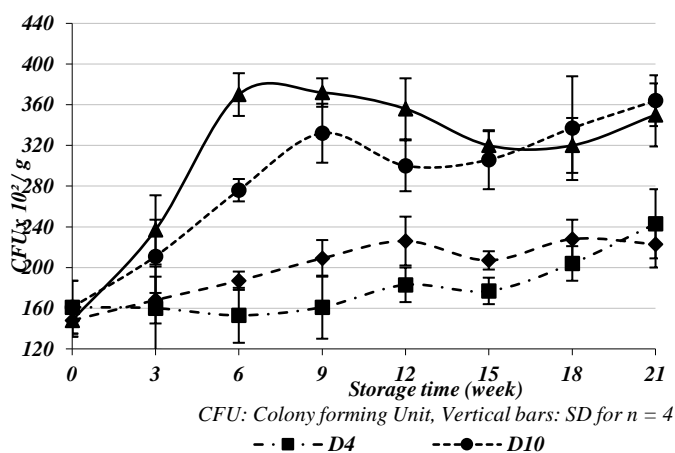


Figure 3 Evolution of total bacteria during storage. The identification and enumeration of pectinolytic *Erwinia*, involving Spunta and Désirée varieties, demonstrated the presence of *Erwinia carotovora* subsp.

*carotovora* (*Ecc*), *E. carotovora* subsp. *atroseptica* (*Eca*) and *E. chrysanthemi* (*Ech*) on tubers of both varieties.

Initially, it can be seen that for both varieties, *Ecc* predominates and represents on average 47% against 36% for *Eca* and 17% for *Ech*. The three species evolve almost in the same way and their multiplication is more important at 10° C than at 4° C, especially on Spunta where *Eca* grows towards the end of the storage period (Fig. 4, Fig. 5 and Fig. 6). Analysis of the results showed that along the storage period, there was a significant differences ( $p < 0.01$ ) between the varieties, and temperatures. During storage, the quantitative distributions of the three species do not undergo a great change and remain in general characterized by the dominance of *Ecc* followed by *Eca*.

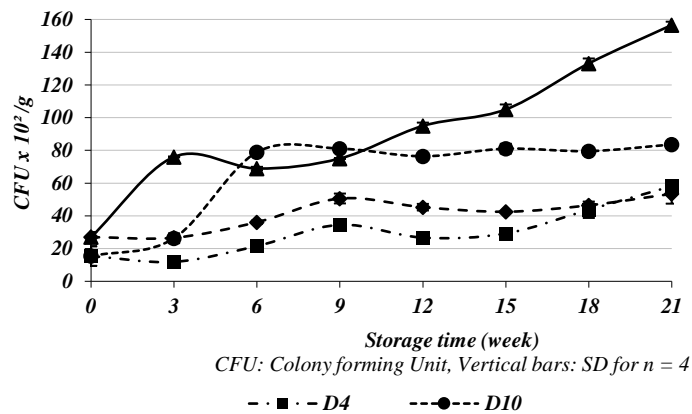


Figure 4 Evolution of *Erwinia carotovora* subsp. *atroseptica* during storage.

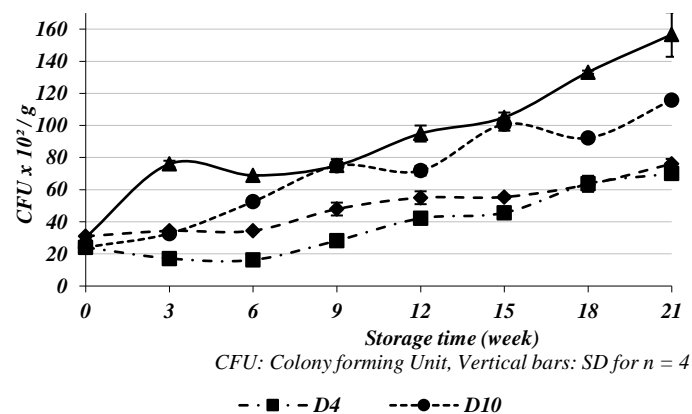


Figure 5 Evolution of *Erwinia carotovora* subsp. *carotovora* during storage.

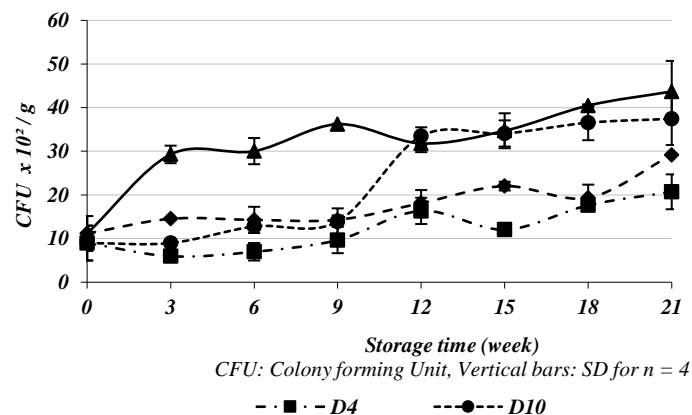


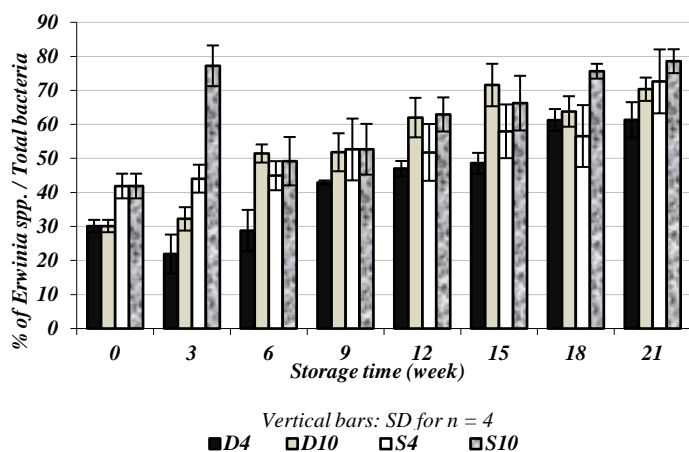
Figure 6 Evolution of *Erwinia chrysanthemi* during storage.

Moreover, it seems that the dominance of one or another species of *Erwinia* varies during storage, which is probably due to the ability of different species to adapt to storage conditions, especially temperature (Horváth et al., 2002; Smadja et al., 2004) and the degree of sensitivity or resistance of the tubers to these bacterial species, which is a varietal characteristic (Morrissey et Osbourn, 1999; Somerhausen, 2003; Reverchon et al., 2016). However, the numerical dominance of *Ecc* is possibly due to its ability to develop better in postharvest by causing soft rots on several stored vegetables (Perombelon et Van Der Wolf, 1998; Laurent

et al., 2001). Furthermore, *Eca* is more active during the vegetative phase than in postharvest storage according to Arias et al. (1998) and Yap et al. (2004). Priou et Jouan (1996) and Helias (1999), attribute blackleg disease of potatoes to *Eca* while, soft rot of tubers in storage is mostly attributed to *Ecc*.

The enumeration of the total bacteria and the pectinolytic *Erwinia* at the reception and during storage highlighted the importance of the variety, which would seem therefore to strongly modify the microbial development on tubers resulting from a same place and a same growing season. It is accepted that for a given variety, the microbial populations in nature and in number, are above all dependent on soil and climatic factors and phytosanitary conditions which prevail in the field (Al-Mughrabi, 2005; Martin, 2004). The effect of low storage temperatures on the development of bacteria on tubers has also been shown to be very remarkable. The proliferation of bacteria in general and pectinolytic *Erwinia* in particular has been shown to be significantly reduced (Larrigaudiere et al., 1987; Shapiro, 1998; Afek et Orenstein, 2001; Soltani et al., 2002).

The assessment of the *Erwinia* spp./total bacteria ratio and its evolution during storage indicated that since the start of storage, *Erwinia* spp. became more important as the shelf life increases (Fig. 7). Initially, all pectinolytic *Erwinia* representing on average 30% of the total bacteria on the Désirée variety against a bit more than 40% on Spunta, this difference confirms again the very significant effect ( $p < 0.01$ ) of the potato variety on the bacterial component of tubers. Along storage, it was the storage temperature that influenced the most and significantly ( $p < 0.01$ ) the importance of *Erwinia* spp. compared to total bacteria. In fact, at the end of storage, the rate of *Erwinia* spp. on the two varieties fluctuated between 60 and 70% at 4° C versus 70 and 80% at 10° C. All these results further corroborate and confirm the importance of pectinolytic *Erwinia* in postharvest and during storage of potatoes in particular and vegetables in general where they develop even under refrigeration conditions and causing crop losses (Rouxel et Jouan, 1999; Elissèche, 1999; Li et al., 2020)



**Figure 7** Evolution of *Erwinia* spp. Rate (*Erwinia* spp. / Total bacteria) during storage.

## CONCLUSION

The results of this study showed clearly that the tubers rot and microbial development, including pectinolytic *Erwinia*, are highly affected by the storage temperature and the potato variety. Whether at 4 or 10°C, the Désirée variety appeared to be better adapted to the studied storage conditions than the Spunta variety. The choice of variety and the management of storage conditions are essential in maintaining the postharvest quality of potato and reduce stock loss. Otherwise, opting for storage at 4 or 10° C is a binding choice, because temperatures close to 10° C ( $\geq 8^{\circ}\text{C}$ ), recommended for the storage of potatoes intended for household consumption and to industrial processing, are not sufficient to prevent sprouting for a long period and slow down the microbial development which results in rotting. However, storage at low temperatures ( $\leq 5^{\circ}\text{C}$ ) often results in biochemical alterations such as low temperature sweetening of potatoes. Nevertheless, storage at low temperatures followed by a reconditioning process or raising the temperature a few weeks before the end of storage is an option to consider.

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