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Influence of Thermal and Pressure Treatments on Inhibition of Potato Tuber Sprouting

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Abstract

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The effect of short duration thermal treatments (60 and 65°C for 1 min) and low intensity high pressure treatments (15 and 30 MPa for 10 min) on the sprouting of potato tubers was applied individually and sequentially, as an attempt to achieve higher inhibitory effects. Thermal treatments only slightly reduced sprouting, evaluated by the number of sprouted tubers, number of sprouts per sprouted tuber, sprout elongation rate, and sprout length. The pressure treatments alone resulted in a slightly higher inhibitory effect compared to the thermal treatments alone. However, it was for the combined treatments when the highest inhibitory effect on sprouting was observed, particularly when potatoes were stored under controlled temperature and humidity conditions that promoted faster sprouting. The combined treatments versus the control led to a much lower number of sprouted tubers (50% vs 100%), number of sprouts per sprouted tuber (4 vs 20), sprout elongation rate (1.48 ± 0.24 mm/day vs 38.5 ± 2.80 mm/day), and sprout length (71 mm vs 1542 mm). These inhibitory effects on sprouting can be of interest and potential for industrial application.

Keywords: high pressure treatments; heat treatments; individual and combined treatments; potato sprouts; sprouting onset; reduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world, being one of the major staple foods, with the majority of its production being used for human consumption (Sonnewald 2001). Tubers are harvested in many countries only once a year and then they are stored for a long time for further industrial processing and fresh market. However, sprouting occurs when dormancy is broken, and the onset of sprouting is influenced by several factors, such as tuber physiological and storage conditions (Owolabi *et al*. 2013). The control of sprouting is essential for potato tuber storage since sprouting leads to alterations in texture, causing softening, shrinkage and formation of toxic alkaloids and consequently reduces the weight, the nutritional and processing quality of tubers and the number of marketable potatoes, being responsible for important economic losses (Sorce *et al*. 2005). The primary methods used to control potato sprouting of stored tubers are storage at low temperatures, the use of chemical sprouting inhibitors, and irradiation (Jadhav & Kadam 1998). However, potatoes for processing cannot be stored at low temperatures for a long time because of the resulting accumulation of reducing sugars. Chemical sprouting inhibitors such as chlorpropham (CIPC, isopropyl 3-chlorocarbanilate) have been used successfully for more than 40 years. CIPC interferes with the cell division

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to inhibit sprouting but the massive indiscriminate use of synthetic chemicals including agricultural chemicals has imposed many problems on the environment and some chemical sprouting inhibitors are banned in some countries or increasingly face restrictions in their use. Other alternatives that may be effective in controlling potato sprouting are under study and include the use of hydrogen peroxide or iodine, ultraviolet-C irradiance, controlled atmospheres with chlorine, low-energy electrons, and genetic modification of tubers (Eolini *et al*. 2004; Kumar *et al*. 2009; Cools *et al*. 2014). These limitations, together with the increasing consumers' demand to eat healthy foods without the use of chemical additives, have been subjected to scientific research for the study of new non-chemical methods to inhibit potato tuber sprouting, which can be consumer and environmentally friendly at the same time.

Heat treatments have been used as non-chemical methods to reduce sprouts and spoilage in potatoes. Ideally, the treatment should supply a lethal dose of heat to assure microbiological safety on the surface and 'cauterise' eyes without damaging the nutritional and processing qualities of the potato tubers (Ranganna *et al*. 1998). The temperature of the water bath and time of immersion are the critical factors of the process and their optimum ranges depend on tuber dimensions (Ranganna *et al*. 1998). High pressure processing (HPP) is an increasingly important physical food technology, which is commercially used to cold pasteurise food products (Ramirez *et al*. 2009). Since pressure influences the metabolism of cells (Sironen *et al*. 2002; Karjalainen *et al*. 2003) and inhibits protein synthesis in bacteria and in eukaryotic cells (Elo *et al*. 2005), it is possible that pressure can influence the sprouting process. HPP can also inactivate enzymes and induce gene expression modification, thus opening a possibility of affecting physiological processes, like potato sprouting (Castro *et al*. 2006; Domitrovic *et al*. 2006). In addition, it is known that pressure influences several physiological and metabolic processes of cells, such as genes, cytokines, and protein expression and causes condensation of the Golgi apparatus and disturbance of the cytoskeletal organisation (Sironen *et al*. 2002; Karjalainen *et al*. 2003). Pressure also inhibits cellular protein synthesis in bacteria and in eukaryotic cells, and might affect cellular processes of potato tubers, which in turn can affect sprouting (Elo *et al*. 2005). According to Eolini *et al*. (2004), the transcription of polyphenol oxidase (PPO) coding genes in potato tubers is responsive to iodine treatments that inhibit sprouting (the inhibition being concomitant with the transient increase in the levels of PPO mRNAs). The regulation of sucrose availability is another possible way to control potato sprouting, by controlling the concentration of the co-factor inorganic pyrophosphate (PPi). Removal of this compound on transgenic potato tubers led to delayed sprouting, but plant growth and tuber development of transgenic plants were severely impaired (Sonnewald 2001).

The aim of this study was to investigate the effect of short duration thermal treatments and/or low intensity high-pressure treatments on sprouting of potato tubers stored under environmental conditions or under conditions that promoted fast sprouting. Both thermal and pressure treatments were applied individually and sequentially combined. Potato sprouting was evaluated by the number of sprouted tubes, number of sprouts per sprouted tuber, sprout elongation rate, and sprout length.

MATERIAL AND METHODS

Potato tubers. Potato (*Solanum tuberosum* L.) tubers of Desiree cv. were planted in June and harvested at the end of August in the Aveiro region and then stored under the same environmental conditions used by farmers to store potato tubers to delay the occurrence of sprouting, without the use of chemical sprouting inhibitors, until the experiments were carried out. Potatoes were stored under the seasonal conditions verified in Portugal: in the first weeks (summer), skin suberisation of tubers takes place and the subsequent colder and higher relative humidity (RH) ambient conditions (autumn/winter) retard sprouting. The first set of experiments (tubers stored under environmental conditions) was carried out at the beginning of December and the second (tubers stored under temperature and humidity controlled conditions – more details further in this section) at the beginning of February, after 3 and 5 months of tuber storage, respectively. Tubers of 25 ± 5 g were chosen for the experiments because tubers of larger size would not fit the pressure vessel used for pressure treatments. Before the experiments six healthy tubers per experiment were thoroughly washed in running water, rinsed twice with distilled water and air-dried.

Pressure and thermal treatments. For pressure treatments, six air-dried potato tuber samples were randomly selected and placed in plastic bags. The air

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was removed from the bags using a domestic vacuum packaging machine (Vacupack 2; Krups, Offenbach am Main, Germany), and the bags were heat sealed. The potato tubers were subjected to the pressure treatments, using an Autoclave Engineers (Erie, USA) isostatic press (Model IP3-23-30), of 15 and 30 MPa for 10 min at room temperature (18–20°C).

For thermal treatments, six potato tubers were vacuum packaged and heat sealed as for the pressure treatments. Potato tubers were submitted to 60°C and 65°C for 1 min in a thermal batch (Tectron Bio 3773100; Selecta, Barcelona, Spain).

Sequentially combined treatments were developed in the same conditions as described above. The pressure was applied before the thermal treatment and the combinations used were 15 MPa (10 min) and 60°C (1 min); 15 MPa (10 min) and 65°C (1 min), 30 MPa (10 min) and 60°C (1 min), and 30 MPa (10 min) and 65°C (1 min). These combinations were selected because previous results obtained by us (Saraiva & Rodrigues 2011) showed that 3 and 5 min of thermal treatment as well as 70 and 75°C resulted in the absence of sprouting, while 1 min at 60 and 65°C only slightly reduced sprouting.

Sprouting experiments. The potato tubers were placed in perforated plastic trays and sprouting experiments were carried out in environmental conditions of temperature and relative humidity. The average, minimum and maximum temperature and relative humidity were 22, 16, and 30°C and 62, 38, and 85%, respectively. To study the effect of the thermal treatment at 60°C (1 min) and 30 MPa (10 min) with sprouting development under environmentally controlled conditions, the experiments were conducted at 19 ± 1°C and 83 ± 2% of relative humidity, in the absence of light. These conditions are in the range of those promoting fast sprouting (Struik & Wiersema 1999) to better ascertain the possible inhibitory effect of the treatments studied. Potato tubers without any treatment (neither treated by temperature nor by pressure) were also placed in perforated plastic trays and sprouting experiments were carried out in the same conditions (control samples).

Tubers were considered to have sprouted when showed at least one sprout with a minimum length of 3 mm. Tuber ability to sprout was evaluated by the percentage of sprouted tubers and the number of sprouts (Saraiva & Rodrigues 2011). Sprout development was assessed by the periodical measurement of sprout length and the determination of sprout length increment rate, by linear regression analysis of the curve relating sprout elongation with time. The sprouting experiments were terminated after 43 days, due to the extensive sprouting of the control tubers. At the end of the sprouting experiments, the total sprout mass was also quantified to ascertain the sprout ability to develop. Six tubers were analysed for each treatment. The rate of sprout elongation was measured by the zero-order kinetics constant using the mathematical description of a zero-order kinetics process (Eq. 1), where A_0 and A are the initial sprout length and the sprout length over time, respectively, k is the zero-order kinetics rate constant, and t is the time (Saraiva & Rodrigues 2011):

$$A = A_0 - kt \quad (1)$$

Visual aspects of potato tubers and their sprouts were registered photographically after 22 days and 57 days of storage under conditions promoting sprouting for each treatment. Specifically, the treatments analysed were 60°C (1 min), 30 MPa (10 min) and 30 MPa (10 min) combined with 65°C (1 min). Control samples (untreated) also were photographed after the same storage period.

Statistical analysis. Statistical significance of the pressure treatments applied was carried out by analysis of variance and Duncan's multiple range test at a 0.05 level of probability.

RESULTS AND DISCUSSION

Figure 1A shows that all combined treatments clearly retarded sprouting compared to the heat treatments alone. At the end of the storage time all combined treatments showed a lower percentage of sprouted tubers (83%) compared to the control and the temperature and pressure independently applied (100%).

The number of sprouts/sprouted tuber was lower for treatments performed at 30 MPa, compared with the individual thermal treatments or control (Figure 1B). At the end of the storage time were observed 15, 10, and 8 sprouts/sprouted tuber for 30 MPa, 30 MPa/60°C, and 30 MPa/65°C, respectively, while 18 sprouts/sprouted tuber were counted for individual thermal treatments and 22 sprouts/sprouted tuber for the control. Combined treatments at 15 MPa resulted in intermediate results between combined treatments performed at 30 MPa and pressure or thermal treatments applied individually.

The total length of sprouts was significantly higher in control tubers (122 mm) and in tubers submitted

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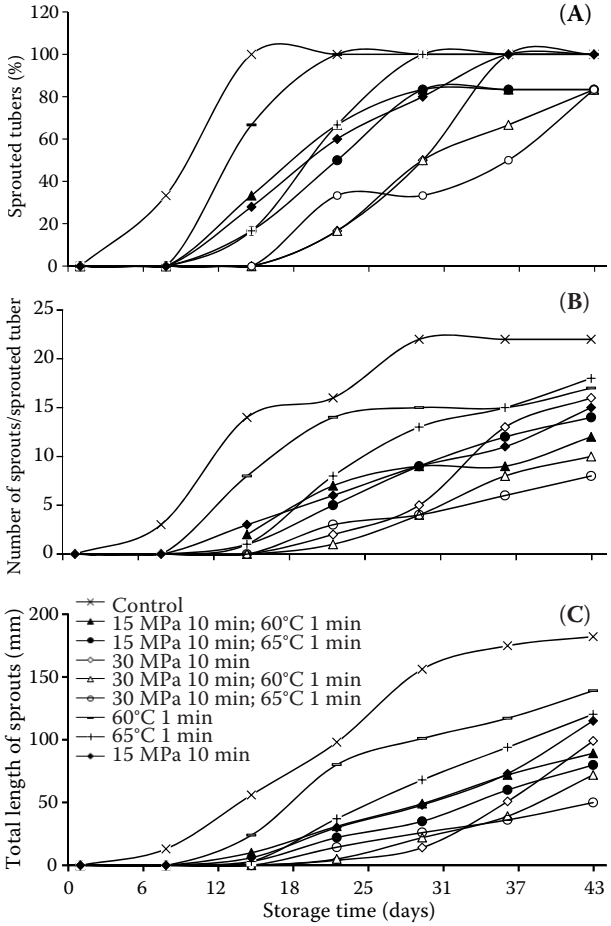

Figure 1. (**A**) Percentage of sprouted tubers, (**B**) number of sprouts/sprouted tuber, and (**C**) total length of sprouts quantified during the storage time, for tubers treated by pressure and thermal treatments

to individual thermal treatments (139 and 121 mm for 60 and 65°C, respectively) than in pressurised tubers (Figure 1C). At the end of storage, the sprouts summed up 99, 72, and 50 mm for 30 MPa, 30 MPa/60°C, and 30 MPa/65°C, respectively, and 115, 89, and 80 mm for 15 MPa, 15 MPa/60°C, and 15 MPa/65°C, respectively. Both pressure levels revealed an inhibitory effect on sprout development when compared with the control or the individual thermal treatments, which presented sprouts with more than 120 mm. The evolution of the length of sprouts occurred linearly, after the initial onset period of sprouting it revealed a zero-order kinetics for sprout elongation. The rate of sprout elongation, measured by the zero-order kinetics constant, was lower for the sequentially combined treatments, with the effect being more pronounced for the combination of 30 MPa and 65°C (1.67 ± 0.07 mm/day) (Table 1). Kinetics constants for individual thermal and pressure treatments as well as control were significantly higher than 3.35 ± 1.11 mm/day (the value obtained for 30 MPa and 10 min). These results indicate a higher inhibitory effect of the sequentially combined treatments on sprouting, by hindering sprout development.

Sprouting development under conditions promoting fast sprouting. The sequentially combined treatments showed higher inhibitory effects on sprouting as quantified by the percentage of sprouted tubers and number of sprouts, compared to the control and the thermal and pressure treatments applied individually and clearly retarded sprouting (Figure 2A).

For the control and the thermally treated tubers, 100% sprouting was observed after only 15 and 22 days of storage, respectively. On the other hand, for combined treatments only 50% sprouting was obtained

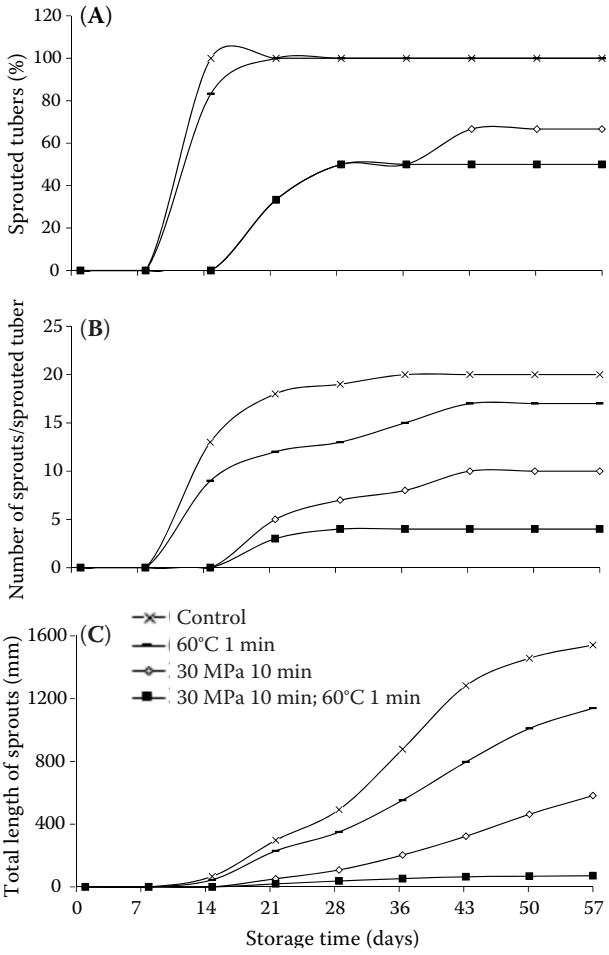

Figure 2. (**A**) Percentage of sprouted tubers, (**B**) number of sprouts/sprouted tuber, and (**C**) total length of sprouts quantified during the storage time under conditions promoting fast sprouting, for tubers treated by pressure and thermal treatments

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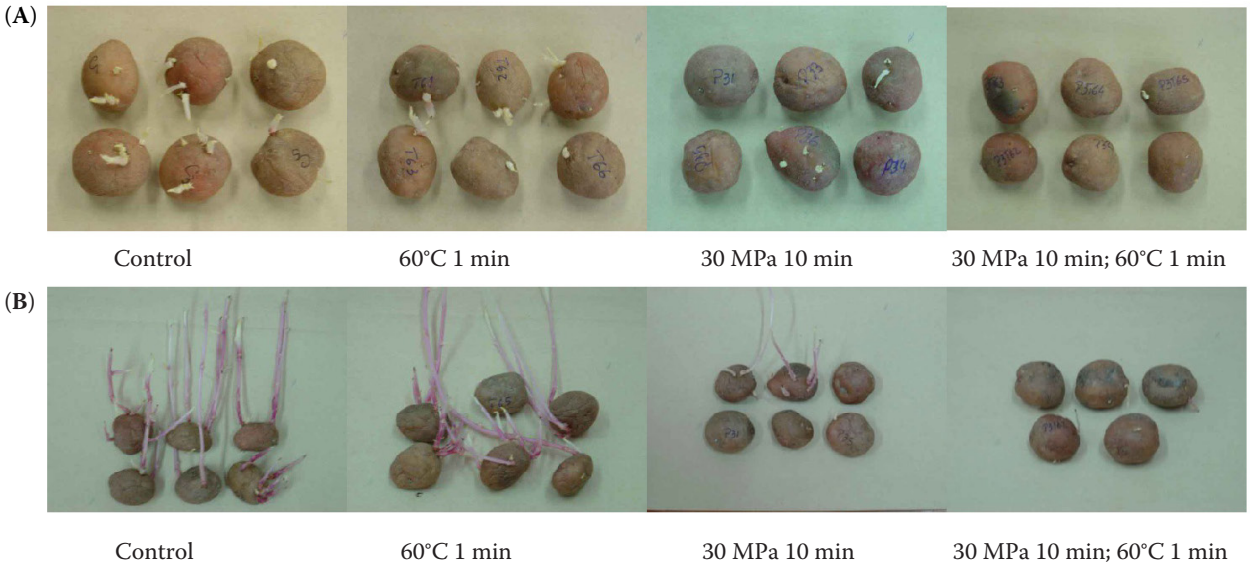

Figure 3. Visual aspect of potatoes tubers and their sprouts after (**A**) 22 days of storage and (**B**) 57 days of storage under conditions promoting sprouting for each treatment

even for the longest storage time considered (57 days). For the combined treatments, the lowest number of sprouts/sprouted tuber (Figure 2B) was also obtained (4 sprouts/sprouted tuber) when compared with pressure applied individually, control or thermal treatments (10, 20, and 17 sprouts/sprouted tuber, respectively). After storage time the total sprout length of 71 mm was observed for the sequentially combined treatment, while for control, thermal and pressure treatments applied individually 1542, 1139, and 582 mm were measured, respectively (Figure 2C). The rate of sprout elongation, measured by the zero-order kinetics constant, was much lower for the sequentially combined treatment (1.48 ± 0.24 mm/day) than for the control (38.5 ± 2.80 mm/day) (Table 1). The retardation of sprouting onset, as well as the lower number of sprouts for the combined treatments are clearly visible (Figure 3). The reasons behind the inhibitory effects caused by the applied treatments are not known. According to Eolini *et al.* (2004), the transcription of polyphenol oxidase (PPO) coding genes in potato tubers is responsive to

Table 1. Rate of sprouts elongation of tubers after pressure and thermal treatments applied individually and sequentially stored under environmental conditions (average, minimum and maximum temperature and relative humidity was 22, 16 and 30°C and 62, 38 and 85%, respectively) or conditions promoting sprouting (19 ± 1°C and 83 ± 2% of relative humidity, in absence of light)

Treatment	Normal sprouting			Fast sprouting		
	n	*k* (mm/dia)*	R^2	*n*	*k* (mm/dia)*	R^2
Control	4	6.74 ± 0.38^a	0.99	7	38.5 ± 2.80^a	0.97
15 MPa 10 min	3	3.72 ± 1.08^c	0.97			
30 MPa 10 min	3	3.35 ± 1.11^c	0.90	6	15.7 ± 1.01^c	0.98
60°C 1 min	5	3.82 ± 0.62^c	0.93	7	27.0 ± 1.02^b	0.99
65°C 1 min	5	4.16 ± 0.15^b	1.00			
15 MPa 10 min; 60°C 1 min	5	2.70 ± 0.28^d	0.97			
15 MPa 10 min; 65°C 1 min	5	2.74 ± 0.15^d	0.99			
30 MPa 10 min; 60°C 1 min	3	2.4 ± 0.04^d	1.00	6	1.48 ± 0.24^e	0.90
30 MPa 10 min; 65°C 1 min	4	1.67 ± 0.07^e	1.00			

*mean ± standard error; *n* – observation number used in *k* quantification; different letters indicate values statistically different (Duncan test, $P < 0.05$)

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iodine treatments that inhibit sprouting (the inhibition being concomitant with the transient increase in the levels of PPO mRNAs). Since it is known that pressure treatments can increase PPO activity in some fruits and vegetables (Mozhaev *et al*. 1996), pressure inhibition of sprouting might be related to an increase of PPO activity. Also, the regulation of sucrose availability by controlling the concentration of the co-factor inorganic pyrophosphate, PPi, (Sonnewald 2001) is another possible explanation for the inhibitory effects observed on sprouting by the combined treatments. Since there are no other studies in the literature concerning the effect of pressure treatments on potato tuber sprouting, it is not possible to compare the results obtained with those of other works.

The inhibitory effect of combined treatments is of interest for industrial application and for fundamental studies but further research is necessary to identify the physiological and metabolic changes behind sprouting inhibition caused by the applied treatments.

CONCLUSIONS

The results obtained show that short duration thermal treatments caused only slightly inhibitory effects on potato tuber sprouting, as well as low intensity pressure treatments, while the sequential combination of these treatments revealed a more pronounced inhibitory effect, as quantified by the evolution of sprouted tubers, number of sprouts, and total length and length increment rate of sprouts. When treatments were sequentially combined and sprouting was studied under favourable conditions, the inhibitory effect on sprouting was clearly more evident. Pressures of 30 MPa tended to lead to a higher inhibitory effect. As the combined treatments applied in this work use short temperature treatments (1 min) and low intensity pressure treatments (15 and 30 MPa for 10 min), no visible injuries were observed in the tubers throughout the storage period (43 days). Thus, the inhibitory effect of these treatments is of interest, potentially allowing for industrial application and for fundamental studies, to control and study the mechanism of breaking potato tuber internal dormancy and sprouting initiation. But more work is necessary to define the optimum combinations and the storage time that can be achieved with sprouting inhibition being of industrial and commercial interest.

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