## QUALITY DECAY AND SHELF-LIFE STUDY OF FRESH CELERY (Apium graveolens L.) GROWN UNDER DIFFERENT NITROGEN FERTILIZATION

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#### **ABSTRACT**

Nitrogen fertilization is useful and widespread to obtain higher growth and quality of productions. To evaluate the influence of nitrogen-rich fertilizers on the quality characteristics, shelf-life tests of celery grown with two levels (80-120 kg ha<sup>-1</sup>) of two different nitrogen fertilizers were performed. Celery plants were selected and packed in two plastic films (anti-fog polyolefin and micro-perforated polypropylene). Nitrate contents, weight loss, hardness, changes in colour parameters and total phenols, were studied in both packaging solutions during storage. Results showed that celery fertilized with organic nitrogen and packed in anti-fog polyolefin reached a shelf-life of 37 days. Not treated sample packed in micro-perforated polypropylene was not marketable after 20 days, while the same sample packed in anti-fog polyolefin reached 30 days.

**Keywords:** Celery; nitrogen fertilization; shelf-life; quality.

#### 1. Introduction

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Celery (Apium graveolens L.) belongs to the Umbelliferae family (sin. Apiaceae), its water content is around 90-96% and it is a hypo-calorific vegetable with only 15-20 kcal/100g of edible portion. In the European Community the main quality standards for celery marketability are reported in the Reg. CE n.1591/87. Freshness, healthiness, cleanliness, but also weight and length ranges of the celeries are clearly described, and products are consequently divided in first and second class. Celery grows up to 30-40 cm tall and is composed of leaf-topped stalks arranged in a conical shape that are joined at a common base. It is a biennial vegetable plant able to grow spontaneously in wetland, and during the second year can develop empty stems up to 60 cm tall with white-green flower. Nitrogen fertilization determines a higher growth of plants. an increase in the leaves system and higher yields. On the other hand, excess nitrogen could cause plant diseases together with an increase in nitrates amount in the edible part of the vegetable (Tei, Natalini, & Bruni, 2000). Consumers awareness of the relationship between foods and health, together with environmental concerns, has led to an increased demand for organic foods. Currently, celery stalks are available in markets as ready-to-eat vegetables, thanks to new packaging solutions which regulate weight loss and gas exchange, keeping freshness and quality longer (Robbs, Bartz, McFie, & Hodge, 1996). Celery washed and chopped in little sticks, with its crunchy texture and distinctive flavour is a popular ingredient in salads and many cooked dishes. The aim of this study was to assess the shelf-life of celery plants as a function of different nitrogen supply in field and of different packaging materials.

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#### 2. Materials and methods

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#### 2.1. Chemicals

53 (N-morpholino)propanesulfonic acid, flavin adenine dinucleotide, microcystein, polyvinylpyrrolidone, mercaptoethanol, etilendiaminotetracetate (all reagent grade), Folin-54 Ciocalteu reagent, catechin (> 98 % purity) and sodium molybdate diidrate were purchased 55 56 from Sigma-Aldrich S.r.l. (Milan, Italy); sodium fluoride, leupeptine and salvcilic acid 57 respectively from Fluka,; Riedel-de-Haen (Hanover, Germany) and Applichem (Darmstadt, 58 Germany). Ethanol and water were analytical grade and were obtained from Lab-Scan (Milan, 59 Italy).

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### 2.2. Plant material, processing and storage conditions

Celery plants var. Dulce cv. D'Elne grown near Catania (Italy), were planted in lysimeter of random squares on a medium composition soil (rich in C and P and pH 7.54). Plants were treated with two different nitrogen fertilizers (ammonium sulphate and organic nitrogen as water soluble peptides). Two levels (80-120 kg ha<sup>-1</sup>) were used, and an untreated square was kept as a control. All tests were performed in triplicate. Samples are referred to as Tn where n is the nitrogen kind and amount used, as reported in Table 1. Sample Rn was grown in a part of the field which had been treated as samples Tn one year before, while it was not treated during this investigation. Celeries were harvested at commercial ripeness and brought to the laboratory. After being carefully inspected, leaves and basal rosette were brushed to remove any soil residues and each sample was weighed, packed and labelled and stored at 4 °C. Two different films commonly used for fresh vegetables were used to pack celery: antifog polyolefin (AF) and micro-perforated polypropylene (MP), both provided by System Packaging s.r.l. (Siracusa, Italy). Non-perforated, antifog polyolefinic film (AF) (Clysar AFG-E, France) is a co-extruded polyethylene and polypropylene film, while micro-perforated film (MP) is a co-extruded polypropylene with a density of perforation of 7 holes/cm<sup>2</sup> (Bemis Le Trait sas, France). Technical characteristics of packaging materials are reported in Table 2. Nitrate contents were monitored in order to correlate the fertilization levels used with the

79 amount of N-NO<sub>3</sub> recovered in field.. The following qualitative parameters were determined

twice a week: nitrate contents, weight loss, hardness and colour on petioles and total phenols. 80

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#### 2.3. Relative humidity

Relative humidity was determined by AOAC method (AOAC, 1990). 83

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#### 2.4. Nitrate contents

Two hundred milligrams of petioles and leaves were ground into powder in the presence of liquid nitrogen containing 50 mM (N-morpholino)propanesulfonic acid in potassium hydroxide, pH 7.8, 5 mM sodium fluoride, 1 µM sodium molybdate diidrate, 10 µM Flavin Adenine Dinucleotide, 1 µM leupeptine, 1 µM microcystein, 0.2 g/g fresh weight of polyvinylpyrrolidone, 2 mM β-mercaptoethanol, and 5 mM etilendiaminotetracetate. The crude homogenate was then centrifuged for 5 min at 12.000g and +4°C. The NO<sub>3</sub><sup>-</sup> concentration was estimated from NO<sub>2</sub> produced after nitrate reduction until a constant concentration in the reaction medium was established. The metabolic nitrate concentration in the tissue was proportional to the total nitrate obtained from the conventional salicylic acid method (Cataldo, Haroon, Schrader, & Youngs, 1975). The complex formed by nitration of salicylic acid under highly acidic conditions has a maximum absorbtion at 410 nm in basic solutions (pH >12). Absorbance of the chromophore is directly proportional to the amount of N- NO<sub>3</sub> present. The nitrate concentration was expressed as mg NO<sub>3</sub> 100 g<sup>-1</sup> fresh matter.

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#### 2.5. Weight loss

Weight loss was determined by weighing all samples with Chyo Balance MK-500C (±0,01 g) 101 102 (Japan) at the beginning and during the storage period. Results were calculated as the difference between the final value and the initial weight and expressed as a percentage.

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#### 2.6. Determination of hardness

Hardness measurement was performed at room temperature (24±2 °C) by measuring the maximum shear force using a Texture Analyzer TR® mod. 53205 (Forlì, Italy), fitted with a stainless steel probe (diameter 5mm). For each celery, three stalks (internal, medium and external) were tested (Rizzo, & Muratore, 2009). Celery stalks were penetrated in two points for each node along each stalk. Data were recorded in duplicate and expressed as N s<sup>-1</sup>.

## 2.7. Colour development

Colour was measured on petioles surface at harvest and during storage using a portable colorimeter (NR-3000, Nippon Denshoku Ind. Co. Ltd, Japan) calibrated with a standard white tile (UE certificated) with illuminant C/2°, (light source used for the daylight), according to CIE Lab scale. Colour was described as coordinates L, a and b (where L measures relative lightness, a relative redness, and b relative yellowness). Colour were measured on the petioles on two points for each node along each stalk. Results were expressed also as hue and chroma ( $C = (a^{*2} + b^{*2})^{1/2}$ ) (Gomez, & Artes, 2004). Hue is one of the three main attributes of perceived colour in addition to lightness and chroma (or colourfulness). Hue values were obtained as  $\underline{h} = \tan^{-1} (b^*/a^*)$  when  $a^*>0$  and  $b^*>0$ , or as  $\underline{h} = 180^\circ + \tan^{-1} (b^*/a^*)$  when  $a^*<0$  and  $b^*>0$ . Data were collected for the petioles as explained for texture analysis.

## 2.8. Determination of phenolic compounds

To determine the amount of the phenolic compounds celery samples were prepared as previously described (Viňa, & Chaves, 2006) and modified as follows. Fresh petioles were chopped and homogenized; then 10 g mixed tissue were treated with 10 ml 96° ethanol for 60 minutes and then centrifuged (Centrifuge DR 15 B, Braun Biotech International, Melsungen, Germany) for 30 min at 13135 g at 10°C. The supernatant was concentrated at reduced

pressure in a rotary evaporator (Laborota 4000-Efficient, Heidolph Instruments, Schwabach,
Germany) until dryness. Total phenols, expressed as mg catechin/100 g dry matter, were
quantified with Folin-Ciocalteu reagent (Singleton, & Rossi, 1965); absorbance readings were
carried out at 760 nm in a spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan).

2.9. Statistical analysis

Data reported are the average of 6 measurements. Results were analysed by means of a twoway analysis of variance (ANOVA), using Tukey test to compare the means. Different letters

#### 3. Results and discussion

indicate significant differences at P≤0.05.

Relative humidity (%) of petioles remained on similar levels (mean value 93.60 ± 0.60) in all samples. In R4 and T2 the highest (94.31 %) and the lowest (92.81 %) values, respectively, were observed (Fig. 1). Data from a survey on the nitrate content of vegetables in Italy and other European countries range from 1700-2400 mg kg<sup>-1</sup> (Santamaria, 2006).

Nitrate contents were increased by nitrogen fertilization showing higher values in T2 (Table 3), having a good correlation with relative humidity (R² = 0,966). Nitrate contents showed always lower values than the toxicity limits of the European Commission Regulation No. 563/2002 which sets limits for nitrate in lettuce and spinach.

Total nitrogen and proteins showed significant difference between our theses, with higher levels in the treatments in comparison with the control. The Protein yield is a value able to evaluate the efficiency of the system to use the nitrogen absorbed. Since the protein content is strictly dependent on the nitrogen coming from the fertilization, the Protein yield shows the absolute value of the capacity of the system to use nitrogen as protein, independently from nitrogen enrichment. Protein yield (% N proteic/N total) showed values ranging from 1.42 to

157 1.55, respectively, in the control and T4 (Table 3). Others values of protein yield were similar between treatments; this indicates a higher protein synthesis in the organic treatment 158 159 compared to the mineral ones, according to higher total nitrogen and nitrate accumulation in 160 T1 and T2. 161 Weight loss is a physiological event in fresh vegetables that can be limited by controlling 162 storage temperature and humidity, but also by using appropriate packaging. Taking into 163 consideration the classification of horticultural commodities according to ethylene production 164 levels made in previous studies, celery should be classified as a vegetable with a very low rate 165 (Gomez, & Artes, 2004). Weight loss in AF packaged celery was lower than 3% while in the 166 samples wrapped up in MP film, as expected, weight loss ranged from 30% to 52% after only 6 days at the end of the storage (Fig. 2). The organic fertilizer supplied to celeries determined 167 168 a shift of the standard weight loss observed for all the samples after 6 days. Both samples T3 169 and T4 showed the beginning of their weight loss after 10 and 13 days, respectively, when packed in AF. AF film reduced weight loss of celery, minimized package condensation and 170 171 extended shelf life to beyond 31 days. In AF, relative humidity of samples did not change and results were correlated with the weight loss ( $R^2 = 0.772$ ). 172 Taking into account the different fertilization applied in soil, the weight loss observed during 173 the storage depends only from package and not from different nitrate contents in the petioles 174  $(R^2 = 0.27 \text{ and } R^2 = 0.22 \text{ respectively for AF and MP}).$ 175 176 As known, hardness is a quality parameter, and it is a critical index of the overall 177 physiological conditions of vegetables, as reported by Vincent, Saunders, & Beyts, (2002). Crispness and crunchiness are attributes of high quality products and are usually the most 178 179 relevant among consumer requirements. However, the distinction between crispy and crunchy 180 behavior is not very clear (Luyten, Plijter, & Van Vliet, 2004). Fillion and Kilcast (2002) 181 stated that crispness and crunchiness are very complex concept containing sound, fracture characteristic, density and geometry of the product. Celery samples gave two kind of results, 182

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for the first dimension 'hardness' and a different answer from panelists according the second dimension probably due to its distinctive fibrousness ('stringy'). Celery was judged as being the crispest product between the six products selected for the experiments (carrot, celery, cucumber, green pepper, Granny Smith and Golden Delicious apples). Untreated samples packed in MP demonstrated a clear loss in hardness of celery petioles, with a shelf-life of 20 days; moreover the same packed in AF kept hardness until 31 days of storage (Fig. 3). This result should be attributed to the performances of the AF film, which minimized the water vapour loss extending the shelf life; indeed, shelf-life of celery is correlated with low weight and relative humidity loss (Bogusława, & Sławomir, 2006). Considering the different theses, celery plants grown under mineral nitrogen evidenced higher softening in the petioles. showing a shelf-life of 31 days against the 40 days reached by samples treated with organic nitrogen. The different behavior between the two nitrogen treatments could be caused by the different amount of nitrates and relative humidity. In fact, in organic treated samples the lowest nitrate content is linked with a higher water content, giving samples a better consistence during storage. Among the different fertilizations (type and levels of nitrogen), samples grown with the highest amount of both kind of nitrogen had the maximum values for hardness of the petioles. Colour is the most evident quality parameter for consumers, reason why hue and chroma changes in petioles were considered (Rizzo, & Muratore, 2009). The hue angle showed a course close to the foretold value, maintaining little variations for all samples (Tables 4, 5). Chroma value decreased during storage, but it was well preserved by the anti-fog packaging, in which T4 and R4 showed the best results. Until 31 days of storage the behaviour of the mineral versus organic treatment was similar, but the organic thesis reached a shelf life of 37 (T4) and 40 (R4). In the micro-perforated film a rapid drop of the "colorfulness" occurred in the first 17 days.

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Total soluble phenols showed values ranging between 4.46 and 6.67 mg catechin 100g<sup>-1</sup> dry matter (Table 3). Total phenols content decreased in all samples after 6 days until the end of shelf-life, with the exclusion of mineral samples T1 and T2, in which these compounds decreased after 10 days of storage (Fig. 4). Phenolic compounds constitute one of the most important groups of natural antioxidants, owing to their diversity and extensive distribution. They possess biological and chemical properties in common: reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, for chelating metallic ions, tendency to self-oxidation and capacity for modulating the activity of some cell enzymes (Viňa, & Chaves, 2006). The phenolic composition of fruits is determined by genetic and environmental factors but may be modified by oxidative reactions during processing and storage, and the mechanisms involve their antioxidant activity and oxidative browning (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Babic, Amiot, Nguyen-the, & Aubert, 1993; Gil-Izquierdo, Gil, Conesa, & Ferreres, 2001; Alasalvar, Al-Farsi, Quantick, Shahidi, & Wiktorowicz, 2005). The action of phenolics as antioxidants is assumed to be beneficial in both foods and human body, where phenolics are oxidized prior to other food constituents or cellular components or tissues. On the other hand, their role as substrates for oxidative browning is probably restricted to foods and is invariably detrimental although, in some instances, it is intentional and essential to the character of the product. During storage, the decrease of these compounds might have positive effects as phenols are the best substrate for polyphenoloxidases (PPO) and peroxidases (POD), enzymes responsible for the cell wall degradation, in some measure confirmed by the correlation between total phenols and hardness values ( $R^2 = 0.43$ ). Although the oxidation of phenolic compounds leads to discolouration, the products are considered as antioxidants. Browning is caused by physiological events, which occur during ripening, or by side-effects of technological processes, involving crushing or wounding. Cross-linked polymers formed by reactions of protein functional groups have been indicated as the browning agents (Robards et al., 1999).

A significant correlation between the amount of total phenols and <u>protein yield</u> was observed in both mineral and organic samples with a slightly higher value in the former ones ( $R^2 = 0.99$  and  $R^2 = 0.92$ ) respectively. Our results are coherent with literature: as previously described by Sitaramaiah and Singh (1978), amendment of soil supplemented with NPK fertilizers increased its phenolic content; as fruits and vegetables from organic cultivation contained significantly more compounds with antioxidant properties (Kazimierczak, Hallmann, Rusaczonek, & Rembiałkowska, 2008; Shevtsova, Nilsson, Gallet, Zackrisson, & Jaderlund, 2005; Bavaresco & Eibach, 1987; Chassy, Bui, Renaud, Van Horn, & Mitchell, 2006).

#### 4. Conclusions

N-NO<sub>3</sub> contents in celery plants were influenced by nitrogen fertilization. Weight loss is a physiological event in fresh vegetables and an important quality parameter that should be considered and studied more to control different factors such as storage temperature, humidity and mainly using the appropriate packaging solution. Sensory quality (colour, texture etc.) of celery is also significantly influenced by growing practices and fertilisation. Particularly, the optimization of irrigation and nitrogen levels has been thoroughly investigated to improve crop quality but also in view of environmental considerations and farm economics (Raffo Sinesio, Moneta, Nardo, Peparaio, & Paoletti, 2006). The use of organic soil amendments has been associated with desirable soil properties including higher plant available water holding capacity (Bulluck, Brosius, Evanylo, & Ristaino, 2002). Clear differences in shelf-life were observed: as expected, MP samples has a shorter shelf-life than AF ones. Celery fertilized with organic nitrogen and packed in anti-fog polyolefin reached a shelf-life of 37 days. Not treated sample packed in micro-perforated polypropylene was not marketable after 20 days, while the same sample packed in anti-fog polyolefin reached 30 days. As expected, the anti-fog polyolefin gave the best results, with the longest shelf-life, limiting the weight loss and

ensuring the best quality and hygienic conditions. Positive effect of the nitrogen fertilization compared to the control were observed, and considering that the shelf-life of samples treated with organic nitrogen is only 3 days longer in AF than in MF, looking at the economical facilities, MF packaging could be recommended. Plants treated with organic nitrogen showed an extending in the weight loss, and giving back a better shelf-life explained by the highest hardness of the celery stalks linked with the highest concentration in antioxidant compound. In conclusion, organic nitrogen supply together with MF packaging could achieve the best result for the environment and for the economic management of agricultural cooperative.

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- 335 59.

336	Figure captions
337	Fig. 1 Relative humidity percentage in petioles of celery
338	Fig. 2 Percentage of weight loss in celery packaged in Anti-Fog (A) and in Micro-Perforated
339	(B) film during storage
340	Fig. 3 Hardness in celery packaged in Anti-Fog and in Micro-Perforated film during storage:
341	(A) Control, T1 and T2; (B) Control, T3 and T4; (C) Control and R4
342	Fig. 4 Total phenols in celery packaged in Anti-Fog and in Micro-Perforated film during
343	storage: (A) Control, T1 and T2; (B) Control, T3 and T4; (C) Control and R4.
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Figure 1 Relative humidity percentage in petioles of celery. C is the control; T1 mineral nitrogen 80 kg ha<sup>-1</sup>; T2 mineral nitrogen 120 kg ha<sup>-1</sup>; T3 organic nitrogen 80 kg ha<sup>-1</sup>; T4 organic nitrogen 120 kg ha<sup>-1</sup>; R4 residual organic nitrogen 120 kg ha<sup>-1</sup>.

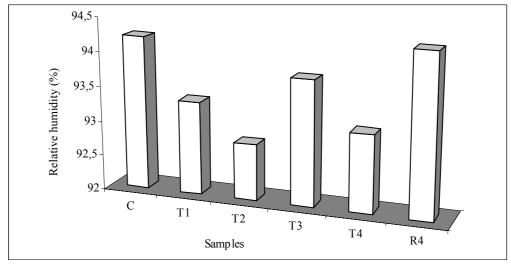
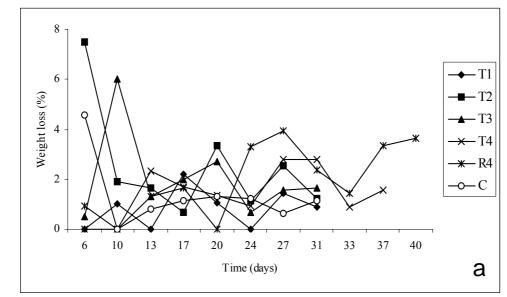


Figure 2 Percentage of weight loss in celery packaged in Anti-Fog (a) and in Micro-Perforated (b) film during storage. C is the control; T1 mineral nitrogen 80 kg ha<sup>-1</sup>; T2 mineral nitrogen 120 kg ha<sup>-1</sup>; T3 organic nitrogen 80 kg ha<sup>-1</sup>; T4 organic nitrogen 120 kg ha<sup>-1</sup>; R4 residual organic nitrogen 120 kg ha<sup>-1</sup>.



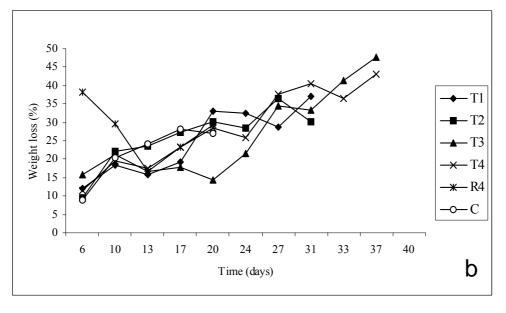


Figure 3 Texture in celery packaged in Anti-Fog (AF) and in Micro-Perforated (MP) film during storage: (a) Control, T1 and T2 mineral nitrogen 80 and 120 kg ha<sup>-1</sup>; (b) Control, T3 and T4 organic nitrogen 80 and 120 kg ha<sup>-1</sup>; (c) Control and R4 residual organic nitrogen 120 kg ha<sup>-1</sup>.

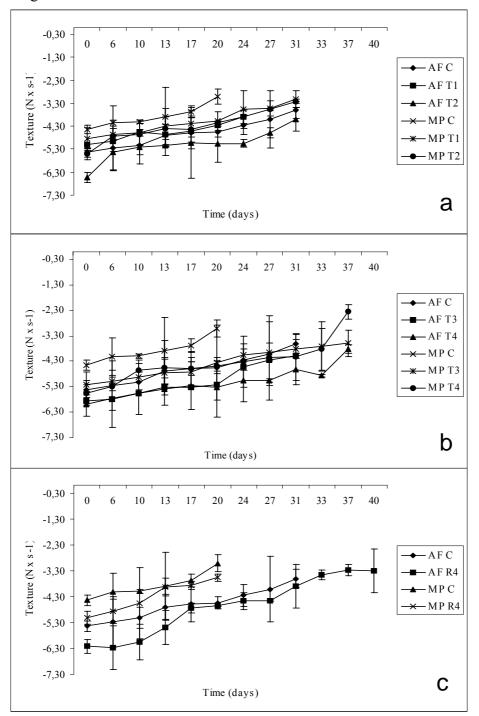
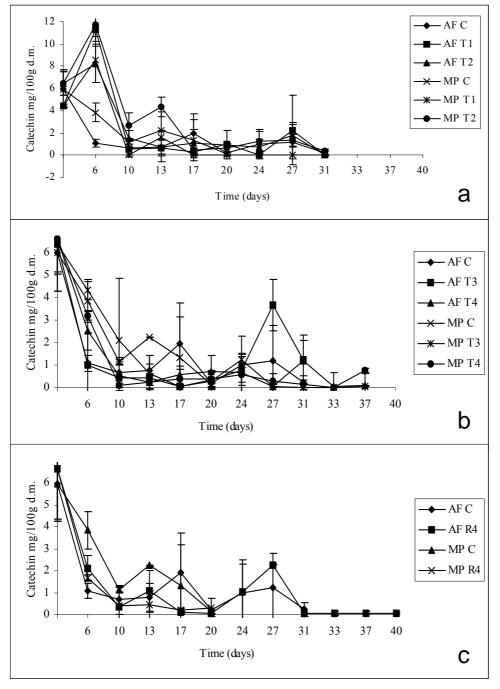


Figure 4 Total phenols expressed as catechin mg content on 100g of dry matter in celery packaged in Anti-Fog (AF) and in Micro-Perforated (MP) film during storage: (a) Control, T1 and T2 mineral nitrogen 80 and 120 kg ha<sup>-1</sup>; (b) Control, T3 and T4 organic nitrogen 80 and 120 kg ha<sup>-1</sup>; (c) Control and R4 residual organic nitrogen 120 kg ha<sup>-1</sup>.



**Table 1.** Treatment, source and quantity of nitrogen applied and N-NO<sub>3</sub> amount in soil.

Sample	Source of nitrogen	Quantity of nitrogen (kg ha <sup>-1</sup> )	NO <sub>3</sub> (mg kg <sup>-1</sup> )
С	-	-	29.64
T1	Mineral	80	100.90
T2	Mineral	120	149.05
T3	Organic	80	37.80
T4	Organic	120	45.39
R4 (last year)	Residual organic	120	34.58

# Table 2. Principal technical characteristics of packaging films (data reported in market schedule).

Properties	Measurements Unit	AF value	MP value
Stiffness Modulus (avg)		193 Mpa	20000 Kg/cm <sup>2</sup>
Thickness	μm	15	25
Elongation	%	150	210
Water Vapor Transmission Rate	$g/m^224h$	24,8	
Oxygen Transmission (PO <sub>2</sub> g/m <sup>2</sup> 24h atm)	$cm^{3}/m^{2}/24h$	14000	
CO <sub>2</sub> Transmission (PCO <sub>2</sub> g/m <sup>2</sup> 24h atm)	$cm^3/m^2/24h$	36000	
Haze	%	3.0	
Gloss	%	110	
Weld ability range	°C		105/150

Table 3. Total nitrogen (N), protein, proteic yield, nitrate (NO<sub>3</sub>) and total phenols in fresh petioles of celery at the beginning of the storage.

Sample	N Protein (g 100g <sup>-1</sup> d.m.) (mg g <sup>-1</sup> f.m.)		Proteic yield (% N proteic/N total)	NO <sub>3</sub> <sup>-</sup> (mg 100g <sup>-1</sup> f.m.)	Total phenols (mg catechin 100g <sup>-1</sup> d.m.)		
С	0,55 e	0,51 e	1,42	247,68 d	4.46 c		
T1	1,25 bc	1,19 c	1,52	518,22 b	6.58 a		
T2	1,61 a	1,54 a	1,53	608,27 a	6.67 a		
T3	1,15 c	1,11 cd	1,51	424,87 c	6.36 ab		
T4	1,45 ab	1,40 b	1,55	486,04 b	6.42 a		
R4 (last year)	1,00 d	0,96 d	1,54	264,55 d	5.96 b		

581 Different letters are significantly different at  $P \le 0.05$ .

621
 622 **Table 4.** Hue in petioles of celery packaged in AF and MP film during storage.

Package	Treatment						Time (	(days)					
1 ackage	Treatment	0	6	10	13	17	20	24	27	31	33	37	40
AF	C	55.22	50.88	51.56	58.15	48.23	51.78	53.87	55.95	50.65	_	_	_
	T1	57.33	53.06	54.07	47.46	52.43	51.78	55.77	51.11	52.85	-	-	-
	T2	58.52	52.00	54.46	51.34	49.23	49.23	51.78	51.11	54.65	-	-	-
	Т3	58.31	50.88	51.11	53.47	56.13	53.87	53.87	58.47	53.06	-	_	_
	T4	53.26	55.03	51.34	50.65	48.74	50.65	51.78	53.47	56.30	54.46	48.99	_
	R4	56.48	52.00	51.56	47.72	52.43	49.23	54.84	53.26	62.48	53.06	54.84	55.22
	С	55.22	57.00	58.47	56.83	56.48	53.26	-	-	-	-	-	-
	T1	57.33	59.68	54.84	53.06	52.64	51.34	54.84	54.26	55.22	-	-	-
MP	T2	58.52	58.31	53.47	58.31	53.67	55.22	55.95	54.46	54.07	-	-	-
IVII	T3	58.31	57.00	54.46	49.72	50.88	53.06	55.95	54.65	53.26	56.65	54.84	-
	T4	53.26	57.66	53.87	56.48	60.39	54.07	54.07	54.84	50.65	57.17	54.65	-
	R4	56.48	60.80	53.87	54.26	50.42	54.65	-	-	-	-	-	-
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**Table 5.** Chroma in petioles of celery packaged in AF and MP film during storage.

Package	Treatment	Time (days)											
1 ackage		0	6	10	13	17	20	24	27	31	33	37	40
	C	22.27	14.75	17.32	12.60	14.66	13.91	17.20	17.55	18.23	-	-	-
	T1	21.31	17.25	12.12	17.36	14.98	17.23	17.16	16.69	19.80	-	-	-
AF	T2	22.08	15.90	16.02	18.69	14.19	14.61	22.47	13.40	15.45	-	-	-
Αг	T3	20.60	18.04	17.41	15.56	15.52	13.98	19.61	14.33	13.79	-	-	-
	T4	20.22	12.89	11.78	18.63	16.17	12.00	13.58	20.10	17.65	28.36	19.40	-
	R4	24.35	20.60	9.90	11.48	13.12	14.90	18.10	19.58	13.70	21.64	15.16	18.64
	С	22.27	24.72	10.28	11.88	10.18	17.18	-	-	-	-	-	-
	T1	21.31	23.08	17.31	12.99	15.09	11.24	17.46	17.45	16.61	-	-	-
MP	T2	22.08	26.16	16.47	6.96	13.28	16.27	17.33	18.29	17.89	-	-	-
MP	Т3	20.60	22.10	15.95	12.60	11.03	20.66	18.38	15.71	16.67	26.99	16.04	-
	T4	20.22	20.24	16.93	12.56	10.04	16.40	16.20	15.61	17.49	25.89	17.57	-
	R4	24.35	15.69	13.01	12.25	13.79	17.49	-	-	-	-	-	-
657													•