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ORIGINAL PAPER

Effect of nitrogen in combination with elevated temperatures on insects, microbes and organoleptic characteristics of stored currants

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Abstract In the present study, the effect of nitrogen, applied as a controlled atmosphere treatment on the microbial and entomological loads, as well as on the organoleptic characteristics of stored dried currants (Corinthian raisins, *Vitis vinifera* L. var. *Apyrena*), was investigated. Trials were conducted under "real world" conditions, in the nitrogen chambers of a commercial facility, in which nitrogen was introduced by using an incorporated nitrogen generator. Prior to the initiation of the trial, chambers were filled with pallets carrying dried black currants. Subsequently, currants were artificially infected with all life stages of *Tribolium confusum*, eggs and larvae of *Ephestia elutella* and adults of *Oryzaephilus surinamensis*. Currants were exposed for 3 days in nitrogen (O_2 concentration <1 %) at two temperature levels, 25 and 38–43 °C. After treatment, insect mortality was recorded and currant samples were collected and forwarded for microbial analysis and determination of their organoleptic characteristics. When nitrogen was applied at 25 °C, high insect mortality levels were noted; however, in most cases there were a number of insects that survived the nitrogen treatment. In contrast, complete control was achieved at 38–43 °C for all insect species and life stages tested, with the exception of *T. confusum* larvae. Nitrogen application at 25 °C had no effect on total microbial and yeast and mould counts, while both were reduced at 38–43 °C. Sensory attributes of Corinthian currants remained acceptable after nitrogen fumigation, although taste, odour, aroma and overall acceptance were affected by the treatments. Total phenolic as well as 5-hydroxymethylfurfural content increased after nitrogen application at 38–43 °C, while the lower temperature applied had no effect. The results of the present study suggest that nitrogen-based controlled atmosphere at elevated temperature could be a valuable tool for ensuring clean, pest-free, hygienic standards in dried Corinthian currants.

Keywords Currants · Disinfestation · Controlled atmospheres · Nitrogen · Sensory characteristics · Stored-product insects

Key message

- Control was complete for most insect species after N_2 fumigation for 3 days at 38–43 °C.
- Total phenolic content was increased after N_2 fumigation for 3 days at 38–43 °C.
- N_2 fumigation had no effect on Corinthian currant appearance and texture.
- Taste, odour, aroma and general acceptance were affected by N_2 fumigation.
- N_2 fumigation for 3 days at 38–43 °C reduced the total microbial and yeast and mould counts.

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Introduction

The use of controlled and modified atmospheres (CA or MA, respectively) is a promising alternative to conventional chemical pesticides for the disinfection and disinfestation of agricultural products (Adler et al. 2000; Banks and Annis 1990; Fleurat-Lessard 1990; Navarro 2012). Taking into account the mounting public awareness about the adverse effects of pesticide residues in food, as well as the environmental concerns about the use of chemical pesticides, the implementation of this technology by the food industry could offer a viable means for securing foodstuff quality during storage. The term controlled atmosphere refers to a modified gas composition in the treated container, produced artificially by the introduction of gases (N_2, CO_2), in order to either reduce the oxygen level or increase the CO_2 concentration (Navarro 2012). The idea of using controlled atmospheres to deal with post-harvest infections and infestations is based on the fact that insects, as well as many microbes, are aerobic organisms that require oxygen for their survival. Therefore, modifying the atmosphere composition within the treated enclosure has a detrimental effect on their development and survival (Butler 2013; Harrison et al. 2006; Hoback and Stanley 2001). The application of controlled atmospheres in storage environment meets several advantages, as it is a non-toxic, environmentally-friendly and residue-free process. Moreover, the mode of action of controlled atmospheres through physiological limitation of respiration renders the development of resistance to this technique by the target organisms highly improbable.

To create a low-oxygen atmosphere, usually nitrogen (N_2) is used (Adler et al. 2000; Navarro 2012); however, other gases, such as helium and argon, have been also successfully tested (Ali Niazee 1972; Lindgren and Vincent 1970). Previous studies with nitrogen controlled atmosphere have showed promising results against storage insects (Bell et al. 1980; Banks and Annis 1997; Donahaye et al. 1996; Ofuya and Reichmuth 1993, 1994, 2002; Navarro 1978; Tunc and Navarro 1983; Hashem et al. 2014). For instance, Ofuya and Reichmuth (1993) evaluated the effect of pure N_2 atmosphere (100 % N_2) on all life stages of the cowpea seed bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), and the bean bruchid, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), and reported complete control of all life stages of both bruchid species within 1–9 days of exposure. Early studies have shown that insects can tolerate low-oxygen levels for long exposure intervals (Bailey 1955, 1956, 1957); therefore, oxygen levels lower than 2 % should be achieved (Adler et al. 2000; Banks and Annis 1990; Fleurat-Lessard 1990; Navarro 1978).

Controlled atmospheres as well as modified atmosphere packaging (MAP) may extend the shelf life of several foods in terms of microbial spoilage prevention or restriction (Sandhya 2010). In MAP, inhibition of microorganisms may be due to direct toxic effects resulting in growth and proliferation inhibition, as in the case of carbon dioxide, or due to indirect inhibitory effects, as in the case of nitrogen. Nitrogen is typically used as an oxygen displacer either alone or in combination with other gases; the gas composition modification alters the ecology of the microbial environment. On this basis, nitrogen has been shown to inhibit the growth of aerobic microbes when applied in MAP (Sandhya 2010). The potential use of nitrogen gas under CA or MAP has been investigated in the case of fresh vegetables and fruits (Ayhan and Esturk 2009; Chara et al. 2012; Koseki and Itoh 2002; Tomás-Callejas et al. 2011) as well as in the case of other food products such as milk (Munsch-Alatossava et al. 2010, 2013), with promising results in several cases. Nevertheless, atmospheres exerting a negative effect on the growth of a particular micro-organism may act as promoters for some others. For example, a high concentration of nitrogen (82 %) or argon and absence of oxygen increased the growth of Lactobacilli species in chicken breast fillets, while at the same time *Pseudomonas* spp. showed a stable growth (Herbert et al. 2013).

Currants (Corinthian raisins) are nutrient-dense dried fruits that are produced from a grape vine variety, i.e. *Vitis vinifera* L. var. *Apyrena*. Southern Greece produces much of the world's supply of currants, continuing a tradition of cultivation in this region for hundreds of years (Chiou et al. 2007). After harvest, grapes are naturally sun-dried in outdoor drying racks. Corinthian currants have high nutritional content, and they are a natural source of hydrophilic antioxidants; therefore, they are considered a food with health promoting properties (Chiou et al. 2007, 2014; Kaliora et al. 2008, 2009; Vasilopoulou and Trichopoulou 2014).

Although the method of controlled atmospheres is well established and its efficacy is well documented, its commercial use for the control of insects and microbes in storage facilities is still limited. Most of the studies showing the efficacy of controlled atmospheres against major stored-product insects in different commodities refer to laboratory bioassays, whereas published information on large-scale nitrogen applications against storage insects is limited. Moreover, no study has been conducted so far to evaluate the effect of nitrogen treatment on currants or Corinthian raisins. Therefore, the objectives of the present study were: (1) to determine the effect of nitrogen-based controlled atmosphere to the microbial flora of currants, (2)

to study the effect of nitrogen-based controlled atmosphere on the organoleptic characteristics, antioxidant content and Maillard intermediate reaction products of currants and (3) to study the effect of nitrogen-based controlled atmosphere for the control of major stored-product insects, such as the cacao moth, *Ephestia elutella* Hübner (Lepidoptera: Pyralidae), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Sylvanidae), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae).

Materials and methods

Test insects and commodity

All insect species used in these trials were reared at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, at 26 °C, 65 % relative humidity (r.h.) and continuous darkness. From the species tested, *T. confusum* and *O. surinamensis* were reared on wheat flour and oat flakes, respectively, whereas *E. elutella* was reared on whole meal wheat flour with 5 % yeast (by weight). For both beetle species, adults <1 month old were used in the tests. In the case of *T. confusum* eggs, larvae and pupae were also used for experimentation, whereas for *E. elutella*, eggs and larvae were tested. All eggs were 1–4 days old, larvae were <7 days old, and pupae were <3 days old.

Untreated and clean Vostizza currants (*V. vinifera* L. var. Apyrena) were used in the trials. Vostizza currant is a Protected Designation of Origin (PDO) subvariety produced in the area of Aegion, Greece. Currants were packed in 10-kg carton boxes and were taken from the 2013 Greek harvest. Currants were inspected for the presence of insects before being used for the experiments and were found to be free of adult or larval stages of insects.

Nitrogen treatment

The trials were conducted in a commercial facility (AgroSpeCom L.T.D., Inofyta, Voiotia, Greece) between January and March 2015. Two sets of trials were conducted inside nitrogen chambers (237.6 m^3), which were sealed and in which nitrogen was introduced by using an incorporated nitrogen generator. High purity nitrogen (99.1 % N_2 and 0.9 % O_2) was produced from air through a pressure swing adsorption (PSA) process and pumped inside the chambers at a maximum flow rate of 35 m^3/h. Temperature and oxygen level in the nitrogen chambers were recorded throughout the trials. Each chamber was filled with eight pallets carrying boxes of packed currants (each pallet contained 64 boxes of 10 kg each).

Plastic cylindrical vials (2.5 cm in diameter, 9 cm in height) were the experimental units for the trials. The vials were perforated in the upper, lower and middle part, and the holes were covered with a US #40 fine mesh screen (0.42 mm openings). The day before nitrogen application all life stages of *T. confusum* (eggs, larvae, pupae and adults), adults of *O. surinamensis*, as well as eggs and larvae of *E. elutella* were taken from the cultures and ten individuals from each insect species and life stage were placed in vials (different vials for each insect species and life stage). In each vial, there were small quantities of flour to allow feeding of the exposed individuals. Vials with insects were placed in two pallets in each chamber (P1 and P2), whereas in each pallet vials were placed in five locations (Fig. 1). Briefly, vials were placed above and under the pallet (L1 and L5), as well as inside the commodity (L2, L3 and L4) (Fig. 1). In order to place the vials inside the product, carton boxes were opened, half quantity of the currants was removed and vials were placed inside the box. Afterwards, vials were covered with the removed currants and the boxes were closed again with sticky tape. There were two vial replicates for each insect species and life stage in each test location. A separate series of six vials from each insect species and life stage was placed outside of the chamber and served as control.

In the first trial, insects were exposed for 3 days to nitrogen atmosphere (O_2 level <1 %) at 25 °C. Similarly, in the second trial insects were exposed for 3 days to nitrogen with temperature ranging between 38 and 43 °C. Each trial was repeated three times. After the termination of the procedure, each nitrogen chamber was opened and all vials were transferred to the laboratory for counting of

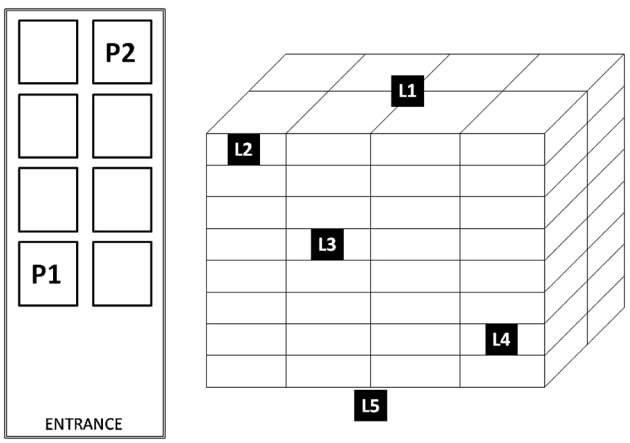

Fig. 1 Distribution of the pallets in the nitrogen chamber (*left*) and of the vials with the insects in each pallet during the trials (*right*). *P1* and *P2* stand for the pallets with insects, whereas *L1–L5* stand for the five locations in each pallet, in which vials with insects were placed

the surviving individuals. Larvae and adults were counted on the next day, while pupae and eggs were placed for 7 days at 26 °C, 55 % r.h. and continuous darkness, to accelerate hatching/emergence. After these 7 days, the vials were opened and all individuals were classified as alive or dead.

At the termination of each trial, samples of currants (approximately 200 g each) were taken for determination of the organoleptic characteristics and analysis of microbial content and phenolic antioxidants. Samples were always taken in duplicate. One of the samples was forwarded for microbial content analysis and the other one for analysis of the organoleptic characteristics and phenolic content of currants. Two untreated samples of currants (approximately 200 g each) served as controls. Samples were stored at room temperature. All analyses were carried out within 1 month.

Corinthian currant organoleptic characteristics were evaluated, before and after nitrogen treatments, by ten trained panellists; appearance, texture (hardness, crystalline texture), taste, aroma, odour and general acceptance of the product were assessed. Sensory attributes were evaluated by hedonic sensory tests and rating tests, on which the sensory panel had been trained. Samples were evaluated by using a nine-point hedonic scale, including point 1 (dislike extremely), 5 (neither like nor dislike) and 9 (like extremely). The product was considered acceptable when rating score was above 5 (Mestdagh et al. 2008). The intensity of sensory attributes was evaluated by a 1–9 rating scale, where 1 was defined as "very slightly/not at all detectable" and 9 as "extremely detectable".

5-Hydroxymethylfurfural (HMF) content was determined after extracting mechanically homogenized currant samples (approximately 1 g) with methanol (2 mL) (Murkovic and Pichler 2006). For the analysis, an HPLC system (Agilent Technologies, model 1050, Waldbronn, Germany) combined with quaternary pump, auto-sampler, diode array detector (HP-1050), and data analysis software was used. RP-HPLC analysis was performed on a Purospher STAR, RP-18 endcapped (250 × 4.6 mm, 5 μm) column (Merck, Darmstadt, Germany) as previously described (Athanassiou et al. 2016).

Phenolic content was analysed as previously described (Chiou et al. 2014); mechanically homogenized currants (approx. 1.5 g) were extracted with methanol/HCl (0.1 % v/v, 4 × 5 mL). Total phenolic content was determined spectrophotometrically (Arnous et al. 2002). All data were acquired using a Specord 200 (Analytik Jena AG, Germany) UV–Vis spectrophotometer. Absorbance was read at 750 nm; results were expressed as gallic acid equivalents (GAE)/100 g currants.

Microbial analyses were performed after preparing appropriate currants dilutions (10^{-1}, 10^{-2}, 10^{-3}) in peptone water; sample homogenization was carried out by Bag Mixer (Interscience, Saint Nom, France) for at least 2 min. Yeasts and moulds determination and total viable count were performed by the pour plate count on Dichloran-Glycerol Agar Base (DG18 CM729 Oxoid, Hampshire, England) and Standard Plate Count Agar (APHA Oxoid CM0463B, Hampshire, England), respectively. Plating was performed in triplicates; the enumeration was based on the average. For yeasts and moulds incubation was conducted at 25 °C for 96–144 h; for total viable counts incubation was conducted at 30 °C for 48 h. Microbial counts were expressed as total number of micro-organisms/g of sample (CFU/g = colonies forming units multiplied by dilution factor).

Data analysis

Control mortality was lower than 13 % for *T. confusum* adults and pupae, *O. surinamensis* adults and *E. elutella* larvae, while control mortalities for *T. confusum* eggs and larvae, as well as for *E. elutella* eggs were higher (ranged between 25 and 82 %). All mortality data from nitrogen-treated insects, with the exception of *T. confusum* and *E. elutella* eggs for which control was complete in all cases, were submitted, separately for each insect species and life stage, to a three-way analysis of variance (ANOVA), with mortality counts as the response variable and temperature, pallet and location as the main effects with the JMP 7 software (SAS Institute Inc., Cary, NC, USA) to indicate if there were differences among treatments. Student's *t* test was performed to compare insect mortalities, separately for each insect species and life stage, obtained at 25 and 38–43 °C ($P < 0.05$) (Zar 1999). In the case of organoleptic characteristics, HMF, phenolic antioxidants and microbial content, results presented are the average of the obtained values. Data handling was carried out using Microsoft Excel and statistical analysis using SPSS (SPSS 20.0 for Windows, Chicago, IL, USA). Organoleptic characteristics were analysed with Kruskal–Wallis test (Mann–Whitney test for post hoc comparisons); statistical significance level was set at $P < 0.05$. For all other parameters studied, one-way ANOVA was applied; Tukey's multiple range tests were performed post hoc to evaluate differences among groups; statistical significance level was set at $P < 0.05$.

Results

Insect mortality

Mortality counts of *T. confusum* adults were significantly affected by all main effects (temperature, pallet and

Table 1 Three-way ANOVA parameters for main effects (temperature, pallet and location) and associated interactions for mortality levels of *T. confusum* larvae, pupae and adults, *O. surinamensis* adults and *E. elutella* larvae

Source	df	*T. confusum*						*O. surinamensis*		*E. elutella*	
		Larvae		Pupae		Adults		Adults		Larvae	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Whole model	19	1.8	0.029	3.3	<0.001	8.1	<0.001	3.1	<0.001	1.1	0.375
Intercept	1	3295.6	<0.001	10,054.2	<0.001	5922.2	<0.001	10,289.1	<0.001	1722.5	<0.001
Temperature	1	2.0	0.166	28.6	<0.001	46.5	<0.001	8.7	0.004	2.7	0.115
Pallet	1	2.1	0.154	0.5	0.498	4.2	0.044	3.7	0.059	1.9	0.182
Location	4	1.4	0.240	2.1	0.085	9.6	<0.001	3.4	0.013	<0.1	0.989
Temperature × pallet	1	0.2	0.636	0.5	0.498	4.2	0.044	3.7	0.059	1.9	0.182
Temperature × location	4	1.4	0.226	2.1	0.085	9.6	<0.001	3.4	0.013	<0.1	0.989
Pallet × location	4	1.6	0.171	1.4	0.255	1.8	0.145	1.4	0.240	<0.1	0.809
Temperature × pallet × location	4	2.9	0.027	1.4	0.255	1.8	0.145	1.4	0.240	<0.1	0.809

location) and most associated interactions (Table 1). Specifically, for *T. confusum* adults complete control (100 %) was achieved at elevated temperature (38–43 °C), whereas at 25 °C average mortality did not exceed 83 % (Table 2). For the same species and stage, significant differences in mortality were recorded among the different locations tested at 25 °C (Table 3). Specifically, the higher mortality levels were observed in L1 (97 %) and L5 (98.9 %) (above and under the pallet, respectively), and were significantly higher than L3 (69.2 %) and L4 (63.3 %), which were both inside the commodity (Table 3). Similarly, for *O. surinamensis* adult mortality was significantly affected by temperature and location, but not by pallet and most associated interactions (Table 1), whereas mortality reached 93.6 and 100 % at 25 and 38–43 °C, respectively (Table 2). Control of *O. surinamensis* adults was complete in three locations (L1, L2 and L5) and significantly higher than L3 and L4 (Table 3). For the rest of the insect species and stages, mortality levels were not significantly affected by most main effects and associated interactions (Table 1). In the case of *T. confusum* and *E. elutella* eggs, complete control (100 %) was achieved in both trials (Table 2). Significant differences were recorded between the two trials for *T. confusum* pupae, for which complete control was achieved at elevated temperature (38–43 °C), whereas at 25 °C, mortality levels reached 89.8 % (Table 2). Finally, in the case of *T. confusum* and *E. elutella* larvae higher mortality levels were obtained at elevated temperature; however, differences between the two trials were not statistically significant (Table 2).

Microbial analysis

Total microbial and yeast and mould counts before and after nitrogen-based fumigation are given in Table 4. In this table, the effect of temperature is presented, given that neither pallet nor location affected the results obtained. A 1.3-log reduction of yeasts and moulds was observed after treatment at 38–43 °C, while total viable count was less affected presenting an approximately 0.4-log reduction. Nitrogen-based controlled atmosphere at 25 °C had no statistically significant effect on either yeasts and moulds or total viable counts.

Table 2 Mean mortality ± SE of *T. confusum* (all life stages), *O. surinamensis* (adults) and *E. elutella* (eggs and larvae) exposed to nitrogen environment in two trials (trial I: 3-day exposure, 25 °C; trial II: 3-day exposure, 38–43 °C) (in all cases $df = 1, 119$)

	T. confusum				*O. surinamensis*	*E. elutella*	
	Eggs	Larvae	Pupae	Adults	Adults	Eggs	Larvae
25 °C	100.0 ± 0.0	92.0 ± 2.7	89.8 ± 2.0*	82.3 ± 3.3*	93.6 ± 2.2*	100.0 ± 0.0	92.2 ± 3.4
38–43 °C	100.0 ± 0.0	96.9 ± 2.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Within each column, means with asterisk obtained at 25 °C, are significantly different from the respective means, obtained at 38–43 °C, according to Student's *t* test ($P < 0.05$)

Table 3 Mean mortality ± SE of *T. confusum* (all life stages), *O. surinamensis* (adults) and *E. elutella* (eggs and larvae) exposed to nitrogen environment in two trials (trial I: 3-day exposure, 25 °C; trial II: 3-day exposure, 38–43 °C) and five different locations (L1–L5) (in all cases $df = 4, 59$)

	T. confusum				*O. surinamensis*		*E. elutella*	
	Larvae		Pupae		Adults		Larvae	
	25 °C	38–43 °C	25 °C	38–43 °C	25 °C	38–43 °C	25 °C	38–43 °C
L1	93.0 ± 6.0	100.0 ± 0.0	98.9 ± 1.1	100.0 ± 0.0	97.0 ± 3.0 A	100.0 ± 0.0	100.0 ± 0.0 A	100.0 ± 0.0	85.1 ± 11.6	100.0 ± 0.0
L2	96.0 ± 1.6	100.0 ± 0.0	84.0 ± 4.8	100.0 ± 0.0	91.0 ± 3.8 AB	100.0 ± 0.0	100.0 ± 0.0 A	100.0 ± 0.0	95.0 ± 5.0	100.0 ± 0.0
L3	92.5 ± 2.8	84.3 ± 10.6	92.5 ± 2.5	100.0 ± 0.0	69.2 ± 5.0 BC	100.0 ± 0.0	85.8 ± 6.1 B	100.0 ± 0.0	92.5 ± 7.5	100.0 ± 0.0
L4	96.7 ± 1.9	100.0 ± 0.0	90.6 ± 2.4	100.0 ± 0.0	63.3 ± 9.9 C	100.0 ± 0.0	85.8 ± 6.5 B	100.0 ± 0.0	92.5 ± 7.5	100.0 ± 0.0
L5	81.0 ± 12.7	100.0 ± 0.0	82.2 ± 8.3	100.0 ± 0.0	98.9 ± 1.1 A	100.0 ± 0.0	100.0 ± 0.0 A	100.0 ± 0.0	97.5 ± 2.5	100.0 ± 0.0
F	1.0	2.2	2.3	–	7.7	–	3.0	–	0.4	–
P	0.398	0.082	0.073	–	<0.001	–	0.026	–	0.836	–

Within each column, means followed by the same upper-case letter do not differ significantly (Tukey's HSD test at $P = 0.05$). Where no letters exist, no significant differences were noted

Organoleptic characteristics

Panellists evaluated the appearance, texture, i.e. hardness and crystalline texture, colour, odour, taste and aroma of currants before and after nitrogen controlled atmosphere application by using a nine-point rating scale (Fig. 2). Nitrogen-based controlled atmosphere fumigation at 25 and 38–43 °C for 3 days did not statistically affect all the sensory attributes evaluated as compared with the control, i.e. untreated currants; among the characteristics studied, taste, odour, aroma and overall acceptance were statistically lower for samples treated under modified atmospheres.

Phenolic compounds and 5-hydroxymethylfurfural

Total phenolic content of the untreated currants was 216 ± 18 mg GAE/100 g ($n = 6$). After nitrogen controlled atmosphere application currant phenolic content found was 196 ± 16 mg GAE/100 g ($n = 6$) and 249 ± 40 mg GAE/100 g ($n = 12$) for trials at 25 and 38–43 °C, respectively. Pair comparison before and after nitrogen application revealed statistically significant differences among trials, with samples treated at 38–43 °C having higher total phenolic content.

Untreated currant HMF content was 14.5 ± 6.9 mg/kg ($n = 6$). After nitrogen application at 25 and 38–43 °C the respective values were 16.9 ± 2.5 mg/kg ($n = 6$) and 37.9 ± 5.8 mg/kg ($n = 12$). The latter value was statistically significantly higher than that of untreated currants as well as of currants treated at 25 °C.

Discussion

The use of nitrogen controlled atmospheres for the control of post-harvest infestations and infections of stored agricultural products has been investigated by many researchers in the past (Banks and Annis 1997; Donahaye et al. 1996; Ofuya and Reichmuth 1993, 1994, 2002; Tunc and Navarro 1983). However, most of the studies have focused on the effect of the method on the sanitary quality of low moisture content durable commodities, such as cereals and legumes. To our knowledge, this is the first study that describes the effect of nitrogen-based controlled atmosphere on the storage insect pests of dried Corinthian currants. Based on our results, nitrogen treatment was highly effective against stored-product insects, at least for the environmental conditions, insect species and life stages tested here. In our study, we used *O. surinamensis*, *E. elutella* and *T. confusum*, as these species are commonly encountered in currant warehouses in Greece, but also in other similar durable commodities (Athanassiou and

Table 4 Total viable counts and yeasts and moulds counts, before and after N_2 treatment at 25 and 38–43 °C for 3 days

Temperature (°C)	Total viable count (CFU/g)		Yeasts and moulds (CFU/g)	
	Before N_2	After N_2	Before N_2	After N_2
25	$3 \times 10^2 \pm 1 \times 10^2$A	$2.2 \times 10^2 \pm 1.4 \times 10^2$A	$3 \times 10^2 \pm 1 \times 10^2$a	$1 \times 10^2 \pm 1.3 \times 10^2$a
38–43	$29 \times 10^2 \pm 12 \times 10^2$B	$13 \times 10^2 \pm 14 \times 10^2$ AB	$37 \times 10^2 \pm 25 \times 10^2$b	$1.8 \times 10^2 \pm 0.9 \times 10^2$a

CFU/g = average plate count multiplied by dilution factor. With respect to total viable counts, values not sharing upper-case letters indicate statistically significant differences at confidence level 95 %. With respect to yeast and moulds, values not sharing lower-case letters indicate statistically significant differences at confidence level 95 %

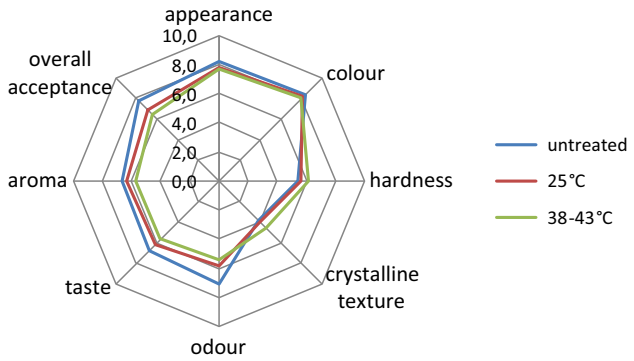

Fig. 2 Sensory acceptability of appearance, colour, texture (hardness and crystalline texture), odour, taste, aroma and overall acceptance of currants, before and after exposure to nitrogen environment in two trials (trial I: 3-day exposure, 25 °C; trial II: 3-day exposure, 38–43 °C)

Eliopoulos 2003, 2004; Buchelos 1980), and high mortality levels were recorded for all three species and life stages. Although insects were kept in vials during the bioassays and were not released inside the commodity, we consider that our experimental design accurately simulates real conditions in storage facilities.

Many studies have highlighted the effect of temperature on nitrogen-based controlled atmosphere fumigation (Chiappini et al. 2009; Donahaye et al. 1994; Soderstrom et al. 1992). All studies suggest that insect mortality is higher at raised temperatures, and have attributed this effect to the speed-up of insect metabolism at higher temperatures (Banks and Fields 1995). For instance, Soderstrom et al. (1992) reported that high temperature (>38 °C) combined with nitrogen-based controlled atmosphere increased mortality of larvae of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and reduced treatment duration. More recently, Chiappini et al. (2009) treated *T. confusum* adults at various O_2 percentages (1–10 %) and temperatures (23–40 °C) and found a negative correlation between the exposure interval and temperature, i.e. that the higher the temperature, the shorter the exposure interval necessary to obtain total insect mortality. A similar effect had been identified in high CO_2 controlled atmospheres, where complete control of a range of storage insects has been accomplished after 1–2 days of exposure at temperatures higher than 38 °C (Jay 1986). The results of our study are in accordance with these findings. When nitrogen application was combined with high temperature levels (38–43 °C), complete control (100 %) was achieved for all insect species and life stages tested, except for *T. confusum* larvae. However, even in this case, mortality of *T. confusum* larvae was considerably high, indicating that *T. confusum* larvae are also highly susceptible to low-oxygen atmosphere and could have been completely controlled with longer exposure intervals. Moreover, the complete control that was achieved for *T. confusum* eggs and pupae, which are considered to be tolerant to stress caused by changes to oxygen level, is indicative of the high efficacy of nitrogen controlled atmosphere at raised temperature. However, what should not be overlooked when designing a disinfestation strategy that involves raised temperatures is the fact that insect thermo-tolerance varies among species, life stage, etc. (Fields et al. 2012). For instance, LT_{99} value for young larvae of *T. castaneum* reached 433 min at 50 °C (Mahroof et al. 2003), whereas 99 % mortality of old larvae of *T. confusum* and *P. interpuncella* was achieved after 90 (Boina and Subramanyam 2004) and 34 min (Mahroof and Subramanyam 2006), respectively. Therefore, depending on the range of the insect species present in a storage facility, the temperature requirements during a nitrogen application may differ.

A drawback of the nitrogen treatment at raised temperatures is the cost of heating the commodities, which can represent a considerable energy cost, especially in northern countries where low temperatures prevail. However, the increased cost will be alleviated by the reduction in treatment time needed. When treating food commodities under high temperatures, concerns may rise over the influence of temperature on the shelf life of the product and its qualitative characteristics. Nitrogen-based controlled atmosphere fumigation had no effect on Corinthian currant appearance and texture (hardness, crystalline texture). Taste, odour, aroma and general acceptance were, however, affected by the treatment. Our results are in line with

those of Guarrasi et al. (2014) and Shamaila et al. (1992), where apple and strawberry sensory quality were affected under MAP as compared with air storage.

HMF is an intermediate Maillard reaction product that is formed by the dehydration of sugars under acidic conditions (Capuano and Fogliano 2011). Although HMF can be formed at ambient temperature, thermal processes are known to drastically increase HMF content (Capuano and Fogliano 2011). In this context, Corinthian currant HMF content was assessed before and after N_2 fumigation treatments. Untreated currant HMF content found in the present study was 14.5 ± 6.9 mg/kg, being in the rather lower limit of dried fruit HMF content reported, i.e. 1–2900 mg/kg (Capuano and Fogliano 2011; Karadeniz et al. 2000; Murkovic and Pichler 2006). Corinthian currant HMF value was also within the ranges (3.6–55.0 mg/kg) reported for dried vine products such as sultanas or Thompson Seedless raisins (Çaglarirmak 2006; Karadeniz et al. 2000; Şevik et al. 2014). Nitrogen application at 25 °C had no effect on the product HMF content, while the content significantly increased at 38–43 °C. In the study of Frank et al. (2004), a significant increase in HMF concentration was observed after storage of *V. vinifera* L. cv. Sultana (Thompson Seedless) raisins for 14 months at 30 °C as compared with storage at 10 °C, hence supporting the present findings. Noteworthy, the HMF value at 38–43 °C, i.e. 37.9 ± 5.8 mg/kg, still remained under the reported ranges for raisin HMF content cited above. Controlled atmosphere treatment for 3 days did not seem to affect HMF formation given that at ambient temperature (treatment at 25 °C) HMF content was practically the same as that of the untreated currants. Therefore, the increase in HMF content observed at 38–43 °C could be attributed rather solely to the temperature increase.

Polar phenolic compounds are ubiquitously present in the plant kingdom. The in vitro ability of phenolics to scavenge free radicals is well established, while evidence from epidemiological and clinical intervention studies is emerging with respect to their protective effects against degenerative diseases, including cancer and cardiovascular diseases (Del Rio et al. 2013). Corinthian currants have been found to contain several simple phenol species and anthocyanins, their total phenolic content being in the range of 150–395 mg GAE/100 g (Chiou et al. 2007, 2014). An increase in the total phenolic content was found after N_2 fumigation at 38–43 °C; this finding is in accordance with the findings of Frank et al. (2004) that also reported an apparent increase in sultana total phenolic content after storage at 30 °C. Such an increase has been attributed to reductones formed via Maillard reactions that together with other species may interfere with the Folin–Ciocalteu assay (Singleton et al. 1985). Additionally, Lee et al. (2003) reported that heat treatment may liberate and activate several low molecular weight natural antioxidants, found in bound forms.

Corinthian currants are usually consumed raw, as snacks; alongside, they are used in bakery and confectionary formulations. In this context, the microbial load is rather of insignificance; however, it becomes crucial under the perspective of expanding currant uses in other edible products, such as dairy products. Nitrogen-based fumigation for 3 days at 25 °C did not affect total microbial and yeast and mould counts. Data on the effect of N_2 atmosphere on the microbial load of dried fruits are scarce. N_2 has been used under MAP for fresh fruits and vegetables preservation with rather contradictory results. The aerobic mesophilic growth on fresh-cut lettuce and cabbage for 5 days at 5 °C and on red chard baby leaves for 6 days at 5 °C was delayed under N_2-enriched MAP, however, analogously to that of passive MAP (Koseki and Itoh 2002; Tomás-Callejas et al. 2011). In ready-to-eat pomegranate arils, the aerobic mesophilic bacteria remained unaltered after 9 days of storage under N_2 atmosphere, while an increase was observed at the end of storage, i.e. 18 days (Ayhan and Esturk 2009). In ready-to-eat arugula salads, N_2-enriched atmospheres combined with H_2O_2 resulted in a reduction in psychrotrophic bacterial load, total mesophilic count and Enterobacteriaceae population after 8 days of storage (Chara et al. 2012). On the contrary, nitrogen was proved ineffective in extending carrot juice shelf life under MAP (Alklint et al. 2004). In our study, a reduction of yeasts and moulds together with a weak effect on total viable counts was observed after treatment at higher temperature, i.e. 38–43 °C. Dried fruits, including raisins, are frequently affected by fungi from *Aspergillus* and *Penicillium* genera (El Halouat and Debevere 1997; Hakobyan 2014) while the osmotolerant Zygosaccharomyces species are the main spoilage yeasts in food with low a_w (El Halouat and Debevere 1996). Most yeasts and moulds are heat-sensitive, being destroyed by treatments at temperatures higher than 60 °C (Jermini and Schimidt-Lorenz 1987). In fact, few *Penicillium* spp. have been reported to grow above 37 °C (Kushner et al. 1979). El Halouat and Debevere (1997) evaluated the effect of water activity, MAP and temperature on the conidial germination of *Aspergillus niger*, *Eurotium amstelodami*, *Penicillium chrysogenum* and *Fusarium oxysporum* isolated from prunes; *P. chrysogenum* and *F. oxysporum* were found incapable to germinate and grow at 40 °C, while *A. niger* germinated and grew better at 30 and 40 °C. On this basis, perhaps the present finding is attributed to the combination of temperatures higher than the ambient with the N_2 environment, although this is a matter of further investigation.

A matter of concern when working with gases is the ability of the gas to overcome any packaging material and penetrate deep enough in the commodity, in order to efficiently kill insects and microbes. For example, ozone, a powerful oxidant that is also used to control insects and fungi in stored grains, rapidly degrades as it moves through the grains and interacts with the grain surface for the first time (Kells et al. 2001; Mendez et al. 2003). At the same time, ozone has poor ovicidal effect (Isikber and Athanassiou 2015). Similar reports have been published for other gases, such as sulfuryl fluoride (Athanassiou et al. 2012; Jagadeesan et al. 2015). The results of the current study showed that nitrogen has a very good ovicidal effect and that, for the species tested, egg was also susceptible to nitrogen. In our study, in order to investigate the penetration ability of nitrogen in the dried currants, vials with insects were placed in various locations inside the nitrogen chamber and in the pallet, i.e. outside but also inside the commodity. At elevated temperature, insect mortality was high in all locations, even in vials placed inside the commodity, where air flow is restricted and oxygen can be easily trapped and create localized limited "oxygen nests". This finding indicates that the reduction in oxygen concentration at the aforementioned conditions was adequate for insect control also inside the commodity and the effect of nitrogen is not location-specific. Similar results, for various exposure intervals have been reported by other researchers, as well as with different experimental protocols (Banks and Annis 1990, 1997; Jay 1986; Navarro 2006). In contrast, at 25 °C, location significantly affected the mortality levels in the case of *T. confusum* and *O. surinamensis* adults, for which control was high in locations outside the treated commodity, but was significantly reduced in locations inside the commodity.

To conclude, nitrogen treatment was highly effective for the range of species examined in this study, at least for the conditions tested. Temperature was identified as one of the key elements that affects the performance of the method, as the increase in temperature resulted in the increase in mortality and the complete control of the insects in most cases. In general, the application of nitrogen against insects was considered as a slow-acting method that usually required long exposure intervals (Navarro et al. 2012). Our study illustrates that by using elevated temperatures, nitrogen can be effective at short intervals (3 days), without any effect on the commodity. This is particularly important, as efficacy at these intervals is usually not achievable by other gases, such as phosphine. Finally, it was shown that nitrogen is remarkably penetrative inside the commodity, i.e. currants, as the efficacy of the method was similar against insects placed inside or outside the commodity.

Author contributions

CGA, AC and VK conceived and designed research. CGA, AC, CIR, SV, MS, EKN, EAP, AK and EK conducted experiments. CGA, AC, CIR and VK analysed data. CGA, AC, CIR and VK contributed to writing the paper. All authors reviewed and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest All authors of this research declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors. This article does not contain any studies with animals performed by any of the authors.

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