Evaluation of carotenoids and furosine content in air dried carrots and parsnips pre-treated whit pulsed electric field (PEF)

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Abstract

Parsnips and carrot slices were subjected to hot drying at 50, 60 and 70 °C with or without pulsed electric field (PEF) pre-treatment at 0.9 kV/cm and 1000 and 10000 pulses. The effect of drying on processed samples was assessed by analysis of carotenoids, the furosine value, total phenols and Whiteness Index (WI). PEF pre-treatment is effective in reducing drying times, but the degree of cellular breakdown makes the compounds more susceptible to chemical and enzymatic reactions. In our condition PEF pre-treatment increased the effect of heat treatment on carotenoid

degradation and promoted the *Maillard* reaction above all at the highest temperature of 70°C. The assessment of carotenoid stability together with furosine value was confirmed to be useful tools for the evaluation of effects of thermal damage and quality of dried food products, also after different drying treatments.

Keywords: PEF, carotenoids, maillard reaction, carrot, parsnip.

Introduction

Dried vegetables, such as carrots and parsnips, are used in ready-to-eat foods, as components of innovative vegetable snacks and instant soups. Parsnip is a root native of Europe and Asia belonging to the Apiaceae family. Parsnip root is known for its health benefits, being part of "white vegetables" with a good source of dietary fibre, having about 30% dry matter [1] (Castro, Bergenståhl & Tornberg, 2012). Therefore, it is related to the control of glycaemia, satiety, and food intake. Carrot is an economically important crop that has gained popularity in recent decades due to the high content of carotenoids, a significant group of biologically active compounds with healthy properties [2, 3] (Melèndez-Martínez, Vicario & Heredia, 2004; Eggersdorfer, and Wyss, 2018) and it is responsible for the colour of a wide variety of foods [4, 5] Panfili, Fratianni, & Irano 2004; Khoo, Prasad, Kong, 2011). Parsnips and carrots are also a good source of health beneficial polyphenols associated with antioxidant activity. The degradation of carotenoids is a common event during thermal treatments of vegetables [6-9] (Saxena, Maity, Raju & Bawa, 2012; Demiray, Tulek & Yilmaz, 2013; Fratianni et al. 2013; Niro et al. 2017). Heating could cause carotenoid losses, in relation to its length and intensity and pre-treatment on various foods [8, 10-12] (Fratianni et al. 2013, 2017, 2018; Donado-Pestana, Salgado, de Oliveira Rios, dos Santos & Jablonski 2012), and could also lead to structural modifications, such as cis-isomerization [13] (Schieber & Reinhold 2005). Based on these reasons, carotenoids could be considered as useful process indicators and as a tool for process optimization.

The Maillard reaction products are widely used as marker of thermal damaged foods. The analysis of furosine generated during the acid hydrolysis of the Amadori compound is one of the most accepted and sensitive method of determining the extent of "early" Maillard reactions [14, 15] (Henle, Zehetner & Klostermeyer, 1995; Erbersdobler, Somoza, 2007), and furosine is considered a marker for the evaluation of various types and conditions of heat treatments [15-18] (Acquistucci, Panfili & Marconi, 1996; Messia, Iafelice & Marconi, 2012; Verardo, Riciputi, Messia, Marconi & Caboni, 2017). The effect of thermal processes on dehydrated vegetable

products is successfully monitored by means of the analysis of furosine [10, 19] (Rufían-Henares, García-Villanova & Guerra-Hernández, 2008; Fratianni et al. 2017). Fruits and vegetables dried with conventional hot air are often treated with sulfur dioxide or sulfite salts as preservantes. There are several reports of sensitivity/intolerance reactions in humans exposed to sulfited solid foods and beverages. Recently the European Food Safety Authority (EFSA) called for the re-evaluation of sulphites used as an additive and concluded that estimate exposure to sulfur dioxide–sulfites was higher than that the adequate daily intake (ADI) of 0.7 mg SO2 equivalent/kg bw per day for all population groups [20] (EFSA, 2016). A way to reduce the use of such preservatives is to use pre-treatments to improve vegetable drying.

Recently, PEF is gaining ground in a non-thermal operation to improve assisted drying. Pulsed electric fields (PEF) processing is a promising innovative technology used to enhance mass transfer during food drying processes [21] (Alam, Lyng, Frontuto, Marra & Cinquanta, 2018). By applying very short electric pulses (1–100 μs), at electric field intensities in the range of 0.1–1 kV/cm, PEF causes permeabilisation due to stress induction in plant cells [22] (Jaeger, Meneses & Knorr, 2014). Moderate-high electric fields (0.1-5 kV/cm) are typically needed for PEF, considering the fact that optimal values depend on the plant tissue and the presence of secondary cell walls [23] (Vorobiev & Lebovka 2008). PEF pre-treatment could significantly save production costs by saving energy. PEF treatment efficiency depends on several factors linked to raw material properties and to treatment conditions. Larger cells are easily electrically damaged, while more resilient and smaller cells have more resistance [24] (Lebovka, Bazhal & Vorobiev, 2002); for instance, in carrots, optimal electric field strength was estimated from 0.2 to 0.4 kV/cm [25] (Bazhal, Lebovka, & Vorobiev, 2003). Only few studies on the combined effect of PEF and convective drying on quality parameters of vegetables are reported in literature [26-28] (Kwao, Alhamimi, Damas, Rasmusson & Gómez Galindo, 2016; Onwudea et al. 2017; Huang et al. 2019). Recently, Alam et al. (2018) [21] investigated the effects induced by a combined PEF pre-treatment and drying on sliced parsnips and carrots. The authors ascertained that the PEF pre-

treatment significantly improved drying efficiency, thereby reducing drying time by 28% in parsnip and 21% in carrot slices, without affecting the texture properties of samples. Still, little is known about the effects of PEF treatment on food quality, as bioactive compound contents and Mailard reaction products formation, especially in case of solid-like matrices. The main objective of this paper was therefore to evaluate the impact on the stability of carotenoids and phenols, and on colour and furosine evolution, on sliced carrots and parsnips treated as in the previous work by Alam et al., (2018), [21], in order to evaluate the effects on thermal damage of conventional e pre-treated PEF in dried parsnips and carrot.

Materials and methods

Raw materials

Parsnips (*Pastinaca sativa* L.) and carrots (*Daucus carota* L.) were bought at a local market and stored at 4°C in darkness before processing.

Pulsed electric field pre-treatment

Parsnip roots (about 200 g) and carrots (about 200 g) were subjected to PEF pre-treatment before drying, by means of a laboratory scale in the PEF unit, with a maximum output voltage of 25 kV (ELCRACK HVP5, DIL, German Institute for Food Technologies, Quakenbrück, Germany). The instrument provides bipolar near-rectangular-shaped pulses: the PEF treatment was performed setting the pulse width of 20 μs, a frequency of 50 Hz and the output voltage of 23%. The output voltage of the instrument was setup at 30%, which gave an electric field strength (E) of 0.9 kV/cm, as recorded in the result section of the instrument after 1000 and 10000 pulses for carrots and parsnips, respectively. Further details on processing procedures can be found in previous works by Alam et al. (2018) [21].

113 Drying experiments

114 Samples (conventional and PEF pre-treated) were sliced into slabs (2 × 25 × 25 mm) and
115 subjected to convective drying at 50, 60 and 70°C, with an air speed of 1 m/s parallel to material,
116 in a laboratory tray drier (Armfield Limited, Ringwood, Hampshire, UK) at different times, in
117 order to in order to reduce the average moisture of parsnip and carrot samples to about 20% of
118 their respective initial values (Alam et al., 2018) [21]. Carrots were subjected to convective drying
119 at 50, 60 and 70°C for 109, 84 and 60 minutes respectively, while were subjected to pre-treatment
120 PEF are dried for 95, 61, 68 minutes respectively. Parsnips samples were subjected to convective
121 drying at 50, 60 and 70°C for 95, 86, and 80 minutes respectively, while subjected to pre-
122 treatment PEF are dried for 95, 82, 57 minutes respectively.
123 Samples subjected to conventional dried samples at three different temperatures (50, 60 and 70
124 °C) are named as CONV50, CONV60 and CONV70, while samples pre-treated with PEF before
125 drying are named as PEF50, PEF60 and PEF70.
126
127 Carotenoid extraction and determination
128 In order to overcome the formation of aggregates during milling, samples were freeze dried by
129 using a freeze dryer Genesis 25SES (VirTis Co., Gardiner, NY), before analysis. Extraction of
130 carotenoids was carried out, in triplicate, through saponification as reported by Fratianni,
131 Mignogna, Niro and Panfili (2015) [29] on 0.5 g of conventional or PEF pre-treated milled
132 samples. The dried residues were dissolved in methanol:MTBE (50:50 mL/mL). Carotenoid
133 extracts were separated by a RP-HPLC system, as reported by Mouly, Gaydou, and Corsetti
134 (1999) [30]. A HPLC Dionex (Sunnyvale, CA), with aU3000 pump and an injector loop
135 (Rheodyne, Cotati), was used. Separation was made at a flow rate of 1 mL/min, under gradient
136 profile, by using a 5 µm C30 YMC (Hampsted, NC, USA) stainless steel column 194 (250×4.6
137 mm i.d.), using Methanol: MTBE: water as mobile phase. A photo-diode array detector (Dionex,

Sunnyvale), set at 430 nm, was used to monitor the eluted compounds. Data were processed by a Dionex Chromeleon Version 6.6 chromatography system (Sunnyvale, CA).

Carotenoid identification and quantification

β-Carotene, α-carotene, 13-cis-β-carotene and 9-cis-β-carotene were compared with known available standards and identified considering their retention times, their diode array spectral characteristics and relative elution order. The α-Carotene, 13-cis-β-carotene and 9-cis-β-carotene standards were obtained from Carote Nature (Lupsingen, Switzerland), while all-trans-β-carotene was obtained from Sigma Chemicals (St. Luis, MO, USA). Purity of all standards was above 95%. Standards were spectrophotometrically quantified and were diluted in methanol:acetone (2:1, v/v) to a concentration range of 5–25 μg/mL. Identified carotenoids were quantified using calibration curves of respective standard solutions. Total carotenoids were the sum of the single quantified carotenoids.

Total soluble phenolic (TSP)

TSP were determined on about 5 g of sample using the Folin Ciocalteau method (Cinquanta, Albanese, Fratianni, La Fianza, & Di Matteo, 2013). [31] The amounts of TSP was determined using a standard curve (0–0.375 mg/mL) obtained with chlorogenic acid (Sigma Aldrich, USA). Data were expressed as mg of chlorogenic acid equivalent (CAE)/kg.

Furosine determination

Furosine content was determined, after hydrolysis, by means of a HPLC procedure, according to Resmini, Pellegrino and Battelli, (1990) [32]. Sep-Pak C18 cartridges (Waters Corp., Milford, MA) were used to purify the hydrolyzates. A HPLC (Dionex, Sunnyvale, CA, USA), equipped with a furosine dedicated column (250 x 4.6 mm, Alltech Italia srl, Sedriano MI, Italy), was used to analyse the samples. The eluted compounds were monitored at 280 nm by a photo-diode array

detector (Dionex, Sunnyvale). Furosine standard was obtained from Neosystem Laboratoire (Strasbourg, France). The analysis was performed in duplicate. Data were expressed as mean values and reported as mg/100g protein.

Whiteness index (WI)

The colour attributes (Hunter L, a, and b values) were measured with a colourimeter Minolta Chroma Meter II Reflectance CR-400 (triple flash mode aperture 10 mm). Each sample was randomly measured at 3 spots. Whiteness index (WI) was calculated according to Patare et al., (2011); [33] and the calculation equation was as the following:

WI: $\sqrt{(100 - L^{*2}) + a^{*2} + b^{*2}}$

Statistical analysis

All drying tests were performed in triplicate and reported data were expressed as means and standard deviations. The analysis of variance (ANOVA) was applied to the data. The least significant differences were obtained using an LSD test ($p< 0.05$).

Linear Discriminant Analysis (LDA) was applied using carotenoids, furosine, total polyphenols and WI as variables for carrots and furosine, total polyphenols and WI for parsnips. Statistical analysis was performed using an SPSS version 20.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results and discussion

The effect of drying at different temperatures on carotenoids in CONV and PEF carrots is reported in Table 1. Figure 1 reports a typical chromatogram of carotenoids in CONV70 and PEF70. The carotenoid profile in fresh carrots showed four principals different identified compounds, in the following elution order: β-carotene, α-carotene, 13-cis-β-carotene and 9-cis-β-carotene. Total carotenoid content was 899.5 mg/kg d.b. (dry basis); the main compound was β-

carotene (75%), followed by α-carotene (14%), 13-cis-β-carotene (9%) and 9-cis-β-carotene (1.5%) (Table 1). In conventional samples, no significant differences in total carotenoid content between control and dried samples and among the different drying temperatures (CONV50, CONV60, and CONV70) were found ($p > 0.05$). For single compounds, no differences were observed for α- and β-carotene. The 13-Cis-β-carotene decreased by about 50% at 50°C and about 40% at 60°C, while 9-cis-β-carotene decreased by about 60%, both at 60 and 70°C. These results are similar to those obtained in previous works on apricots and goji fruits [10, 11] (Fratianni et al. 2017; 2018). Differences between CONV and PEF samples were observed, with a significant reduction of total carotenoids in PEF samples to about 30%, 20% and 30% at 50°C, 60°C and 70°C, respectively. In particular, in PEF50, α-carotene, β-carotene and 9-cis-β-carotene were 22%, 32% and 42% lower than CONV50, respectively, while for 13-cis-β-carotene, no differences were found. PEF60 samples resulted in the same total and single carotenoid contents of CONV60, with the exception of α-carotene and β-carotene, whose amounts were about 15% and 25% lower than in analogous conventional samples. In PEF70, all carotenoids undergo significant losses, about 30% lower than in CONV70. Information concerning the effect of electric fields on bioactive components of food matrix is quite limited. The paper by [34] Kumar, Bawa, Kathiravan and Nadanasabapathi (2015), on mango nectar, reported a reduced carotenoid content after severe PEF treatments, indicating that carotenoid degradation occurred with respect to pulse width increase and to frequency. In a work on carrots by [35] Wiktor et al. (2018), higher total carotenoid values were observed when the plant tissue was treated with lower electric field intensity (1.85 kV/cm), while they decreased after a more severe application of PEF (3 kV/cm). Our results suggest that electroporation of cell membrane could make carotenoid compounds more vulnerable to degradation. Moreover, PEF treatment could promote different reactions in the tissue, causing a decrease in carotenoid content, according to the events occurring during electroporation, such as the formation of reactive oxygen species [36] (Nuccitelli, Lui, Kreis, Athos & Nuccitelli, 2013). The combined treatment of PEF with hot drying could further increase

the degree of cellular structural damage, as also observed by [23] Vorobiev and Lebovka(2008), thus enhancing the effect of thermal treatments on carotenoid degradation. These data were supported by preliminary microstructure observations in which dried PEF samples evidenced the presence of larger cavities than conventional ones (data not shown). A very low concentration of carotenoids was found in parsnips, thus, for these samples, the effect of thermal treatments on carotenoids was not evaluated.

For a complete evaluation of the effects of the drying processes on carotenoids, it is necessary to have a suitable method for their extraction and quantification able to evaluate their real content, regardless of the matrix, water content, their bond to the food components. In several papers, these analytical aspects are underestimated, since the authors reported increases of compound contents during processing. For example, in a recent work, Huanget al. (2019) [28] found in PEF processed apricots a β-carotene content of 130.2 ug/g d.w., in comparison of 94.20 ug/g d.w. in fresh apricot, stating that the observed increased content may be attributed to improvement in the extraction, following electroporation of cell membrane caused by PEF treatment. However, these increases are not likely to be true increases due to technological treatments, but could depend on the fact that processed samples can have a greater extractability from the food matrix and comparison with unprocessed samples is difficult if a sole solvent extraction is performed. Furthermore this greater extractability could not be related to nutritional aspects that should be investigated after bioavailability and bioaccessibility studies. In light of these considerations, our analytical method, by using saponification of the matrix, through alkaline hydrolysis, followed by solvent extraction, should be applied for the exact evaluation of real contents before and after technological treatments, in case compounds are present in forms bound to food matrix, where the application of the sole extraction method would not be sufficient to determine their exact amounts.

Results on furosine determination in PEF and conventional dried carrots and parsnips are reported in Fig.1. Low amounts of furosine were detected in fresh samples (about 12 and 10 mg/100g protein, in carrots and parsnips, respectively). The amounts of furosine in the analysed

242 samples depended on both PEF treatment and the time-temperature combination used during
243 drying. Furosine amount showed a stronger increment in CONV carrots than in CONV parsnips.
244 Moreover, in all PEF samples, an increase in furosine was found. PEF parsnips showed less
245 furosine content than PEF carrots, probably due to a reduction of electroporation effectiveness in
246 parsnips, because of the difficulty in breaking their cell walls, as previously detected (Alam et al.
247 2018). [21] Moreover, the Maillard reaction efficiency depends on different factors, including
248 chemical composition (e.g. amino acids and reducing sugars), temperature, pH, time, water
249 activity and reactant concentration [19, 37] (Rufían-Henares, García-Villanova & Guerra-
250 Hernández 2008; Lertittikul, Benjakul & Tanaka 2007).

251 In parsnip, sucrose is the predominant sugar while the content of glucose and fructose is much
252 smaller (about 0.45-0.75%), with a ratio reducing sugar to non-reducing sugar of about 1/10 (Ilić
253 & Sunić, 2015). [38] Instead, in carrots, higher glucose and fructose contents were found (about
254 15%) (Soria et al. 2010)[39]. PEF pre-treatment could have significantly favoured the Maillard
255 reaction as also demonstrated by [40] Wang, Guan, Yu, Yuan & Xu (2011). The use of other
256 Maillard reaction markers could describe these phenomena more completely [41] (Wellner, Huettl
257 and Henle, 2011).

258 Whiteness index (WI), shows in table 4, represents the overall whiteness of food products that
259 may indicate the extent of discoloration during the drying process [42] (Hsu et al. 2003). For
260 carrots and pastinaca conventional drying resulted in a higher WI than the PEF combined with hot
261 drying. The data suggests a higher browning in PEF sample as already shown by results on
262 furosina. This browning could be avoided with the use of sulphites as in Huang et al, [28] which
263 can block these phenomena, but international indications (EFSA) suggest a reduction of these
264 additives in food.

265 In dried parsnips, a significant reduction in total soluble phenolic (TSP), about 30% was found
266 compared to dried CONV samples. In PEF parsnips, a significant decrease was not found (Table
267 2). According to Caetano and Leal (2006), [43] carrots were classified as “low phenolic content”

vegetables (<100 mg catechin equivalents/100 g fresh weight). Dried carrots showed a similar behaviour with parsnips, with a TSP reduction of about 25% in samples dried at 50°C; whereas, Kroehnke et al. (2018) [44] found a reduction of 42% in total phenolic content after convective drying at 45°C. Unlike parsnips, in carrots, PEF pre-treatment caused a further reduction in TSP, about 20%, compared to CONV samples, regardless of the used temperature (Table 2). Different tissue structure, variety, maturity stage and other differences may be contributing factors. In particular, as previously observed (Alam et al. 2018), [21] a reduction of the electroporation effectiveness in parsnip could be a good explanation for the results obtained.

To better visualize differences among treatments a linear discriminant analysis (LDA) was performed on all samples of parsnips (Figure 3a) and carrots (Figure 3b) using all variables (total phenols, furosine and WI for parsnips and total phenols, furosine, WI and total and individual carotenoids for carrots). In both figures the first function, explained 83 % and 96 % of the total variance, was given by furosine, while the second function was given by WI. The LDA analysis selected the following variables able to discriminate the treatment: furosine and WI for parsnip and furosine, WI, 13cis-βcarotene, βcarotene, 9cis-βcarotene for carrot. These variables, in both cases, are able to clearly separate the samples based on the different treatments.

Conclusions

Drying conditions, combined with PEF pre-treatment had a significant effect on the carotenoids and total phenols content. Moreover, in our conditions they promoted Maillard reaction, evaluated by furosine value, leads a higher browning and phenol and carotenes reduction. Only in the application of PEF pretreatment and drying at 60 °C these phenomena are mitigate, so that it can be considered a good compromise between the reduction of drying time and the preservation of bioactive components. Therefore drying conditions combined with PEF pre-treatment must be appropriately modulated and evaluated in order to avoid negative effects on the final quality of the products. In fact the degree of cellular breakdown due to PEF treatments, makes the compounds more susceptible to chemical and enzymatic reactions. The obtained data also confirmed

carotenoids and furosine as adequate markers in evaluating the drying process, providing information about the thermal damage and the quality of dried vegetables, thus helping in the control and optimization of drying conditions.

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Table 1 Carotenoids (mg/Kg d.b.) in fresh, PEF pre-treated (PEF) and conventional (CONV) dried carrots.

Samples	13-cis-β–carotene	α-carotene	β-carotene	9-cis-β–carotene	Total
Fresh	81.4 ± 4.6 [a]	127.1 ± 3.3 [a]	677.6 ± 50.9 [a]	13.4 ± 0.0 [a]	899.5 ± 52.1[a]
CONV50	41.4 ± 2.6 [b]	139.3 ± 4.8 [a]	684.0 ± 47.0 [a]	11.7 ± 0.9 [b]	876.4 ± 67.1 [a]
CONV60	50.0 ± 1.9 [c]	139.0 ±13.4 [a]	753.8 ± 59.4 [a]	4.4 ± 0.4 [d]	947.1 ± 136.2 [a]
CONV70	73.7 ± 1.3 [d]	139.2 ± 5.0 [a]	690.2 ± 63.1 [a]	6.9 ± 0.6 [c]	909.9 ± 78.7 [a]
PEF50	38.7 ± 4.2 [b]	108.3 ± 5.5 [b]	464.1 ± 14.2 [b]	6.4 ± 0.6 [c]	617.5 ± 23.9[b]
PEF60	47.5 ± 3.8 [c]	118.0 ± 11.5 [b]	562.2 ± 56.5 [c]	5.8 ± 0.1[d]	733.5 ± 78.0 [a]
PEF70	49.5 ± 5.6 [c]	93.4 ± 5.4 [c]	486.0 ± 57.9 [b]	5.0 ± 0.4 [d]	633.8 ± 75.3 [b]

Different letters within the same column indicate a significant difference ($p< 0.05$)

Table 2 Total soluble phenols (TSP) (mg catechin /kg) in fresh, PEF pre-treated (PEF) and conventional (CONV) dried carrots and parsnips.

Samples	Carrots	Parsnips
Fresh	883 ± 35 [a]	473 ± 41 [a]
CONV50	664 ± 29 [b]	381 ± 28 [b]
CONV60	693 ± 35 [b]	355 ± 20 [b]
CONV70	626 ± 44 [b]	340 ± 18 [b]
PEF50	521 ± 32 [c]	393 ± 28 [b]
PEF60	569 ± 17 [c]	398 ± 21 [b]
PEF70	512 ± 22 [c]	352 ± 34 [b]

Different letters within the same column indicate a significant difference ($p < 0.05$)

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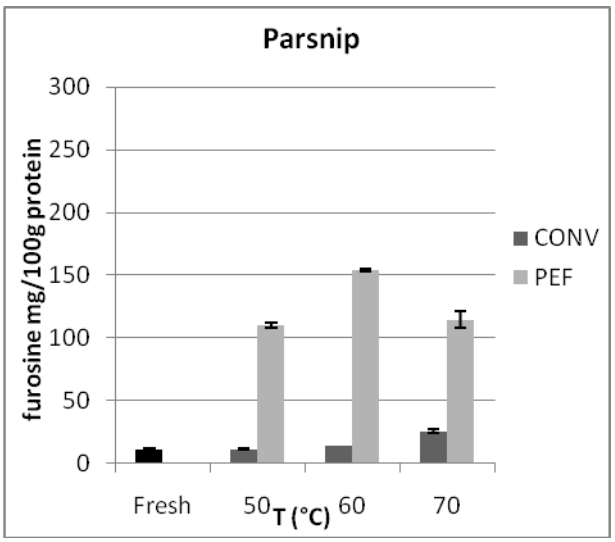

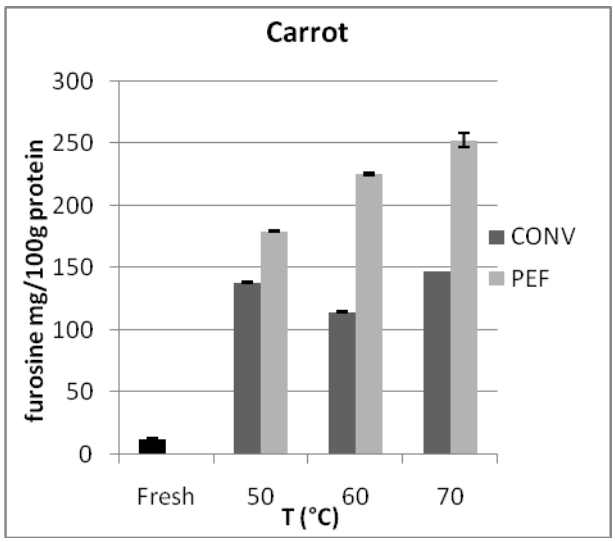

Figure 2-Furosine values in fresh, PEF pre-treated (PEF) and conventional (CONV) dried carrots and parsnips.

Table 3-Whitness index (WI) in fresh, PEF pre-treated (PEF) and conventional (CONV) dried carrots and parsnips

CARROT		WI
	Fresh	41.2
	PEF50	43.7
	CONV50	47.6
	PEF60	41.9
	CONV60	46.0
	PEF70	39.7
	CONV70	47.3
PARSNIP		
	Fresh	62.1
	PEF50	69.5
	CONV50	74.8
	PEF60	70.5
	CONV60	74.6
	PEF70	70.4
	CONV70	77.0

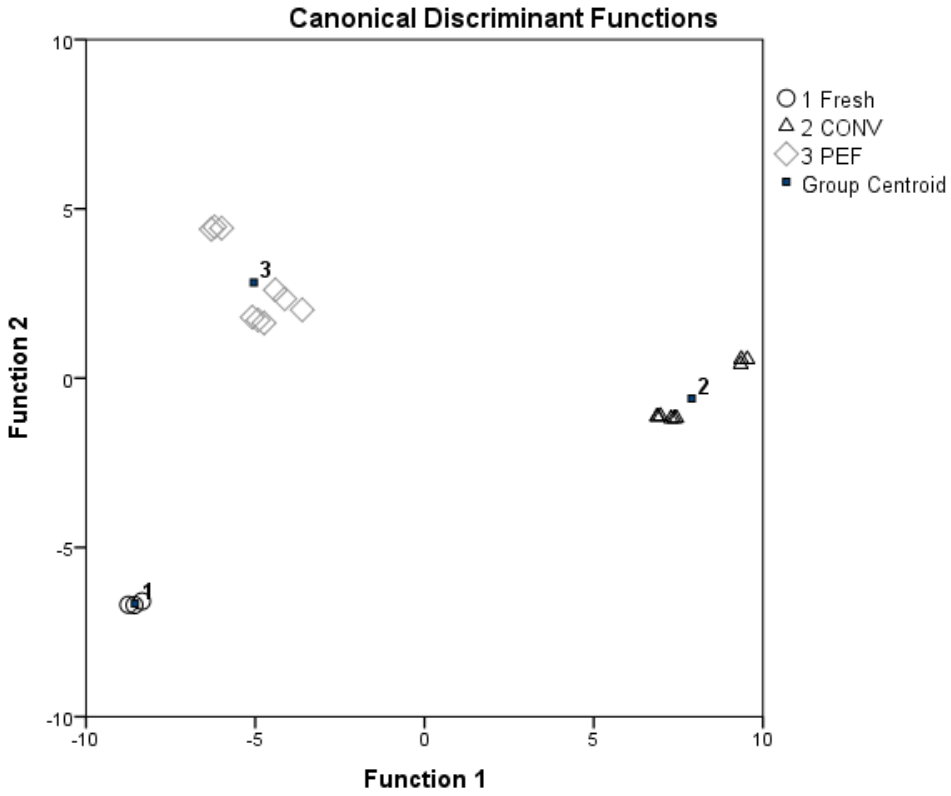

Fig. 3a. Linear discriminant analysis of the different treatments used in parsnip samples.

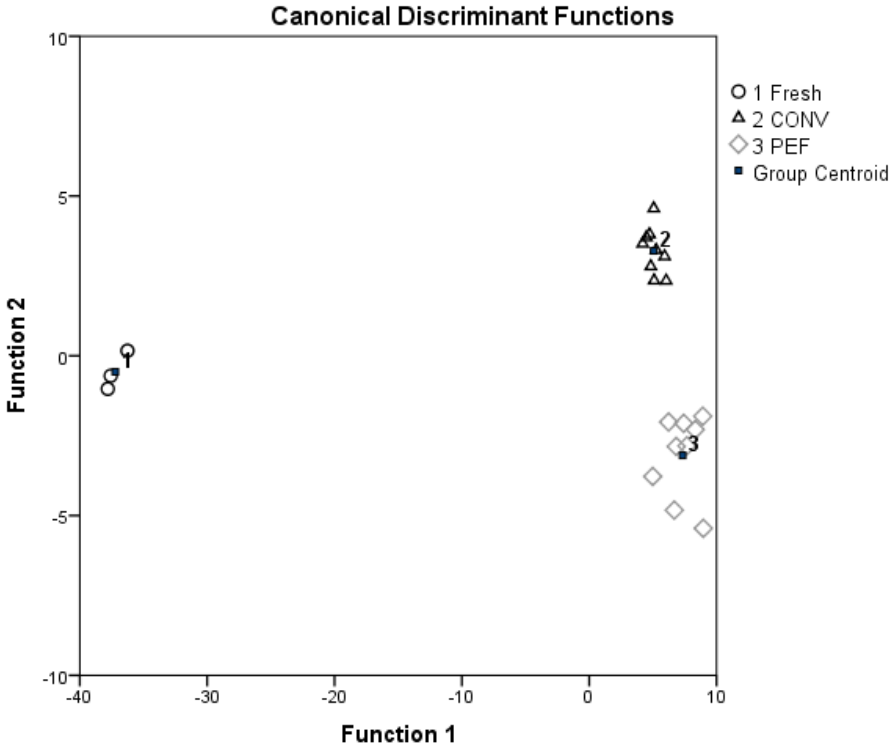

Fig. 3b. Linear discriminant analysis of the different treatments used in carrot samples.

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