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EFFECT OF OIL FLUSHING WITH NITROGEN ON THE QUALITY AND OXIDATIVE STABILITY OF COLD-PRESSED RAPESEED AND SUNFLOWER OILS

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ABSTRACT

Background. Oxidative stability means resistance to oxidation during purchase, processing and storage and is a key quality indicator of edible fats. Oils ought to be stored in dark-glass bottles, at low temperatures and with no access of light in order to effectively preserve their oxidative stability. Since all vegetable oils contain unsaturated fatty acids that can react with oxygen and deteriorate over time, displacement of oxygen with inert gases may result in a reduction of the rate of oxidation. In the study the effect of oil flushing with nitrogen on the quality and oxidative stability of cold-pressed rapeseed and sunflower oils was determinate.
Material and methods. Commercial samples of cold-pressed rapeseed and sunflower oils were stabilized by generating anaerobic atmosphere in the bottles by blowing through with nitrogen and generation of a "nitrogen cushion". Oils were tested in accelerated at 63°C and long-term at 20°C storage tests.
Results. After 20 days of Schaal oven test, the peroxide value in the flushing with nitrogen rapeseed and sunflower oils was, respectively, 4 and 7 times lower than in the control samples (without nitrogen). In turn, of the long-term storage test (with access of light 20°C), the peroxide value of oil flushing with nitrogen after 6 months of storage was 2.3 to 2.8-fold lower, respectively, than in the control sample. In the oil samples flushed with nitrogen peroxide formation was inhibited, however, as a result of the breakdown of the peroxides already existed in the oil, gradual decrease of the oxidative stability (determined via Rancimat test) was observed along with prolonged storage of oils.
Conclusions. Oil flushing with nitrogen was a very effective way to reduce the changes caused by oxidation in cold-pressed rapeseed and sunflower oil.

Key words: cold-pressing, rapeseed oil, sunflower oil, flushing, nitrogen, storage, oxidative stability

INTRODUCTION

Oxidative stability means resistance to oxidation during purchase, processing and storage and is a key quality indicator of edible fats. Among the external factors, the rate of oxidation is affected by the access of oxygen and light, by the presence of pro-oxidative metals, dyes, phospholipids, mono- and diacylglycerols or products of thermal oxidation, as well as by time and temperature of storage (Choe and Min, 2006). In the case of refined oils, the most significant is chemical oxidation which proceeds without the presence of enzymes, whereas in the case of cold-pressed oils and storage of oil materials – important is also biochemical oxidation (Drozdowski, 2007).

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Likewise nutritional value, the oxidative stability of lipids depends on the chemical composition of oils: fatty acid composition and the presence of anti- and pro-oxidative concomitant compounds and interactions between them (Ayton et al. 2012; Choe and Min, 2006; Wroniak and Łukasik, 2007; Wroniak et al., 2006). In terms of the oxidative stability, extra virgin olive oil is followed by rapeseed oil (Koski et al., 2002), that is characterized by a lower content of polyenoic fatty acids and higher oxidative stability (by ca. 30 to 50%) than sunflower oil (Wroniak and Łukasik, 2007; Wroniak et al., 2006).

Ensuring a high quality of the raw material and the course of technological process is insufficient to preserve a high and stable quality of cold-pressed oil throughout its shelf life. Improper post-production handling of oil, inappropriate pouring to packages, the use of an inappropriate package or even a bottle cap, and finally negligence of recommended conditions of storage, distribution and retail may waste the effects of all previous treatments (Matthäus, 2012; Matthäus and Brühl, 2003). As indicated by Ayton et al. (2012) storage of olive oil under poor conditions (storage at high temperature, with the access of light and oxygen) may reduce shelf life of olive oil from 36 to 6 months, due to exceeded IOC limits (The International Olive Council), specified for peroxide value and spectrophotometric indices (K_{232} and K_{268}). Oils – preferably earlier flushed with nitrogen to remove oxygen – ought to be stored in closed, dark-glass bottles, at low temperatures and with no access of light in order to effectively preserve their oxidative stability.

Oxidation proceeding in oils may also be inhibited by the application of various methods decreasing air content in the package, including inert gases: nitrogen and carbon dioxide, and various methods of oxygen elimination, e.g. oil flushing with a specified gas and/or formation of a gas cushion above the oil in the package. These methods ensure significant reduction of oxidation. Both, nitrogen and carbon dioxide, were shown to be highly effective in stabilizing refined oils (Marciniak-Łukasiak et al., 2006; Sionek et al., 2013). Nowadays, oil flushing and spraying with nitrogen are commonly used in the edible oil industry to slowdown the rate of oil auto-oxidation during storage. It yields significant benefits including elongation of induction time in the oxidation process, which allows extension of storage stability of oils and fats (List et al., 2005).

An overview of literature data revealed that, apart from extensive works concerning studies on storage of *extra virgin* olive oil (Cecchi et al., 2010; Gambacorta et al., 2004; Méndez and Falqué, 2007; Sacchi et al., 2008), there are only sparse reports regarding to the other oils, e.g., sunflower (Shafqatullah et al., 2011; Tasan et al., 2011), rice (Mezouari and Eichner, 2007) and practically none related to cold-pressed rapeseed oil.

In this study, the effect of oil flushing with nitrogen on the quality and oxidative stability of cold-pressed rapeseed and sunflower oil was examined.

MATERIAL AND METHODS

Oil samples

Commercial samples of cold-pressed rapeseed and sunflower oils were obtained from Primus (Warsaw, Poland) directly after pressing. Colourless glass bottles of capacity 26 mL were filled to ¾ of their volume. Bottles were tightly closed with a rubber stopper and sealed with aluminium caps.

During storage, both control and flushed with nitrogen oil samples were divided into two series and subjected to different storage tests: (a) Schaal oven test; (b) long-term storage test.

Overall, 208 bottles were considered per each type of oil (16 bottles for each day of scheduled determinations), sampling and analyses were carried out in triplicate on two different bottles for each type of oil, using always unopened bottles.

Nitrogen flushing procedure

The oil flushing with nitrogen (Nitrogen 5.0, BOC Gazy, Warsaw, Poland) was performed using a glass capillary with a flow rate of 5 mL/s during 20 s for each bottle. Nitrogen cushion was created by introducing inert gas into headspace, and thus anaerobic atmosphere was formed within bottles. Control oil samples were not flushed with nitrogen.

Schaal oven storage stability test

Oil samples were submitted to an accelerated storage test which was conducted in forced-draft oven at 63°C

for up to 20 days. Quality indices were determined in fresh oil, and after 4, 8, 12, 16, and 20 days.

Long-term storage test

Bottled samples of oils were stored on the lab shelf exposed to daylight (day-night), at room temperature, where the average temperature during storage period was 20°C (ranging from 18 to 22°C) for up to 180 days. Quality indices were measured in fresh oil, and after 30, 60, 90, 120, 150, and 180 days.

Quality indices

At fixed intervals oil samples were withdrawn, and quality indices were determined according to the following ISO standard methods: acid value (AV) expressed in mg KOH · g^{-1} of oil (ISO 660:2005), peroxide value (PV) expressed in miliequivalents of active oxygen × kg^{-1} of oil (ISO 3960:1996), anisidine value (ISO 6885:2008) and Totox index (PN-93/A-86926).

Rancimat test

Oxidative stability was measured with a Rancimat apparatus (Metrohm model 743; Switzerland). Oil samples were weighed (2.5 g) into the reaction vessel in triplicate and heated to 120°C under air flow of 20 L/h. The induction period (IP) was expressed in hours (h).

Statistical analysis

All experiments were carried out in triplicate. To examine the effect of oil flushing with nitrogen on the cold-pressed oils variables studied, one-way ANOVA was used when the variables fulfilled parametric conditions, and the Kruskal-Wallis test when these were nonparametric (Statistica v. 12 software, StatSoft, Inc., Tulsa, USA). Significant differences between means were determined through Tukey's Multiple Range Tests. P values less than 0.05 were considered as statistically significant. Additionally, data were subjected to Principal component analysis (PCA) and hierarchical cluster analysis (HCA). To explore the correlations among the concerned variables, the Pearson correlation analysis was performed and the results are shown in Table 3 and 4.

RESULTS AND DISCUSSION

The quality characteristic of the examined oils

Fresh cold-pressed rapeseed oil was characterized by lower AV (1.1 mg KOH × g^{-1}), compared to sunflower (1.7 mg KOH × g^{-1}; Table 1, 2), nevertheless the AV of oils did not exceed the value specified in the Codex Alimentarius (≤4 mg KOH × g^{-1}) for cold-pressed and virgin oils (Codex..., 2011). The average value of the peroxide value PV of fresh oils ranged from 1.8 meq O_2 × kg^{-1} for rapeseed oil to 5.3 meq O_2 × kg^{-1} for sunflower oil (Fig. 1, 2), and thus the level of primary oxidation products was within the Codex Alimentarius limits (*PV* < 15 mEq O_2/kg). Low peroxide values could explain negligible changes of essential fatty acids observed during storage. Secondary oxidation products in the oils such as high molecular weight saturated and unsaturated carbonyl compounds were measured as anisidine value (AnV). Cold pressed oils were characterized by very low AnV, which ranged from 0.4 to 0.7 (Table 1, 2).

The EU has established no specific regulations for cold-pressed and *virgin* oils regarding AnV, nevertheless requirements pertaining to the AnV < 8 for refined oils has been specified in the Polish Standard (PN-A-86908:2000). The content of secondary oxidation products in the inspected oils was similar to that reported by Matthäus and Brühl (2003) and Wroniak (2012). Rancimat method is based on the changes in conductivity produced by volatile organic acids which are stable tertiary-reaction products from the oxidised oil. The induction period (IP) is defined as the period during which no oxidative, volatile components are generated under certain, defined conditions. There is a correlation between IP and oil stability – the longer the IP, the more stable oil. As shown in Figure 3 and 4, the IPs of the testing oils showed a significant range of oxidative stabilities, from 2.3 h for sunflower oil to 3.9 h for rapeseed oil. This observation is in agreement with the literature data (Wroniak, 2012; Wroniak and Łukasik, 2007; Wroniak et al., 2006). The IP length differences of the examined oils can be partially explained by the FA composition. Sunflower oil had higher amount of PUFAs (70% of C18:2) compared to rapeseed oil (20% of C18:2 and 10% of C18:3) (Dubois et al., 2007). Auto-oxidation of oils is a complex process initiated by free radical reactions

Table 1. Quality parameters of oils subjected to Schaal oven test

Storage time days	Rapeseed oil – control	Rapeseed oil – flushed with N_2	Sunflower oil – control	Sunflower oil – flushed with N_2
		AV, mg KOH/g		
0	1.1 ±0.0^a	1.1 ±0.0^a	1.7 ±0.0^a	1.7 ±0.0^a
4	1.1 ±0.0^a	1.2 ±0.1^a	1.6 ±0.2^a	1.7 ±0.0^a
8	1.3 ±0.1^a	1.2 ±0.1^a	1.6 ±0.1^a	1.6 ±0.1^a
12	1.1 ±0.0^a	1.1 ±0.0^a	1.6 ±0.1^a	1.6 ±0.1^a
16	1.2 ±0.1^a	1.2 ±0.1^a	1.7 ±0.0^a	1.7 ±0.1^a
20	1.2 ±0.1^a	1.2± 0.1^a	1.6 ±0.0^a	1.5 ±0.1^a
		AnV		
0	0.4 ±0.0^a	0.4 ±0.0^a	0.7 ±0.0^a	0.7 ±0.0^a
4	3.6 ±0.3^b	1.8 ±0.0^b	2.8 ±0.1^b	1.5 ±0.0^b
8	4.5 ±0.1^c	2.5 ±0.0^c	2.9 ±0.0^b	1.5 ±0.0^b
12	6.2 ±0.1^d	2.5 ±0.2^c	5.4 ±0.1^c	2.1 ±0.1^c
16	7.6 ± 0.3^e	3.4 ±0.1^d	6.5 ± 0.1^d	2.3 ±0.0^c
20	8.7 ±0.1^f	4.6 ±0.0^e	7.6 ±0.2^e	3.4 ±0.1^d
		Totox index		
0	4.06 ±v 0.2^a	4.06 ±0.2^a	11.31 ±0.5^a	11.31 ±0.5^a
4	30.92 ±2.5^b	16.46 ±2.5^d	68.05 ± 2.9^b	12.54 ±1.0^b
8	35.92 ±3.5^c	9.66 ±2.1^b	74.58 ±2.0^c	12.94 ±1.4^b
12	40.07 ±2.4^d	14.45 ±2.1^c	76.77 ±3.2^c	19.32 ±1.9^c
16	46.51 ±2.2^e	9.69 ±1.8^b	78.83 ±3.0^d	14.81 ±1.2^d
20	46.38 ±2.2^f	12.82 ±1.8^b	78.96 ±3.9^d	15.31 ±1.2^e

Different superscript letters within each column indicate significant differences ($P < 0.05$).

involving unsaturated fatty acids. Thereby, oils containing unsaturated fatty acids are highly susceptible to oxidation (Choe and Min, 2006; Drozdowski, 2007).

Effect of oil flushing with nitrogen

The study demonstrated a lack of statistically significant ($P > 0.05$) hydrolytic changes, i.e. changes in the AV in the analysed rapeseed and sunflower oils, both in control samples and these flushed with nitrogen, in the accelerated (Schaal oven test) and long-term storage tests (Table 1 and 2, respectively). Irrespective of the test applied, intensive undesirable changes were observed in the rapeseed and sunflower oils upon oxidation along with storage time, but only till the moment of complete consumption of oxygen dissolved in oil or present in the space above oil surface. Owing to a high content of polyenoic fatty acids sunflower oil was oxidizing substantially more rapidly than the rapeseed oil did, which was confirmed in both Schaal oven test (Table 1, Fig. 1) and long-term storage test (Table 2, Fig. 2). The cold-pressed rapeseed oil was characterized by almost 2-fold higher oxidative

Table 2. Quality parameters of oils subjected to long-term storage test

Storage time days	Rapeseed oil – control	Rapeseed oil – flushed with N_2	Sunflower oil – control	Sunflower oil – flushed with N_2
		AV, mg KOH/g		
0	1.1 ±0.1^{a}	1.1 ±0.1^{a}	1.6 ±0.0^{b}	1.6 ±0.0^{b}
30	1.1 ±0.1^{a}	1.1 ±0.1^{a}	1.4 ±0.1^{a}	1.4 ±0.1^{a}
60	1.3 ±0.1^{a}	1.2 ±0.1^{a}	1.3 ±0.0^{a}	1.4 ±0.1^{a}
90	1.2 ±0.1^{a}	1.1 ±0.1^{a}	1.5 ±0.1^{ab}	1.3 ±0.1^{a}
120	1.1 ±0.1^{a}	1.1 ±0.1^{a}	1.3 ±0.1^{a}	1.3 ±0.1^{a}
150	1.1 ±0.0^{a}	1.1 ±0.1^{a}	1.5 ±0.1^{ab}	1.5 ±0.1^{ab}
180	1.1 ±0.0^{a}	1.1 ±0.1^{a}	1.3 ±0.1^{a}	1.3 ±0.1^{a}
		AnV		
0	0.4 ±0.0^{a}	0.4 ±0.0^{a}	0.7 ±0.0^{a}	0.7 ±0.0^{a}
30	1.0 ±0.0^{b}	1.4 ±0.1^{b}	0.8 ±0.0^{a}	0.7 ±0.1^{a}
60	1.3 ±0.0^{b}	1.4 ±0.0^{b}	0.9 ±0.1^{a}	0.7 ±0.0^{a}
90	2.6 ±0.2^{c}	1.4 ±0.1^{b}	1.8 ±0.0^{b}	0.7 ±0.1^{a}
120	3.1 ±0.0^{d}	1.6 ±0.3^{c}	2.0 ±0.1^{b}	0.8 ±0.1^{a}
150	3.4 ±0.4^{d}	1.6 ±0.2^{c}	2.5 ±0.0^{c}	1.0 ±0.2^{b}
180	4.5 ±0.1^{e}	1.8 ±0.2^{d}	3.1 ±0.1^{d}	1.2 ±0.1^{b}
		Totox index		
0	4.06 ±0.2^{a}	4.06 ±0.2^{a}	11.31 ±0.5^{a}	11.31 ±0.5^{a}
30	37.69 ±2.1^{b}	8.09 ±1.4^{b}	53.99 ±3.9^{b}	12.77 ±1.2^{b}
60	50.69 ±2.5^{c}	11.95 ±1.2^{c}	73.75 ±2.8^{c}	15.96 ±1.2^{c}
90	57.47 ±2.1^{d}	18.47 ±1.2^{d}	77.26 ±2.7^{d}	17.31 ±2.4^{d}
120	63.19 ±2.2^{e}	19.91 ±1.5^{e}	81.29 ±3.2^{e}	23.90 ±2.7^{e}
150	69.19 ±2.3^{f}	29.76 ±1.7^{f}	84.86 ±2.4^{f}	30.60 ±1.2^{f}
180	76.59 ±3.2^{g}	31.24 ±1.4^{g}	87.72 ±2.3^{g}	32.31 ±1.8^{g}

Different superscript letters within each column indicate significant differences ($P < 0.05$).

stability in the Rancimat test compared to the sunflower oil, determined both at the beginning and at the end of the tests (Fig. 3 and 4).

Oil's flushing with nitrogen turned out to be a very effective means of inhibiting oxidation in the analysed oils. It was observed that in the accelerated test (no access of light, but high temperature of 63°C), the flushing of oils with nitrogen before bottles closing almost completely inhibited the process of oxidation, as indicated by no increase of primary oxidation products PV (Fig. 1). After 20 days, the PV value in the flushing with nitrogen rapeseed and sunflower oils was, respectively, 4 and 7 times lower than in the control samples. In turn, in the long-term storage test (with access of

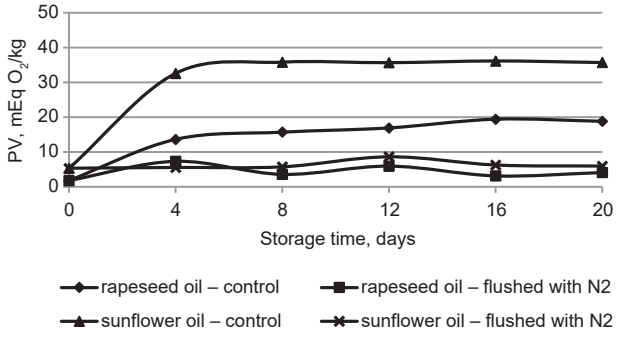

Fig. 1. Peroxide value of the examined oils in Schaal oven test

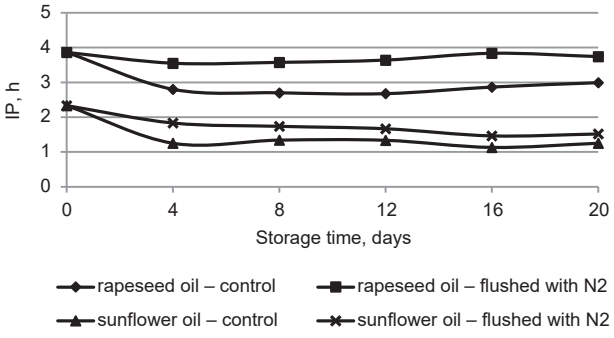

Fig. 3. Decrease of IP [h] during 20 days of storage in Schaal oven test

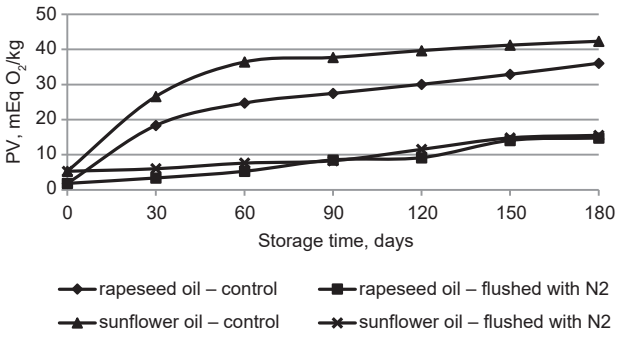

Fig. 2. Peroxide value of the examined oils in long-term storage test

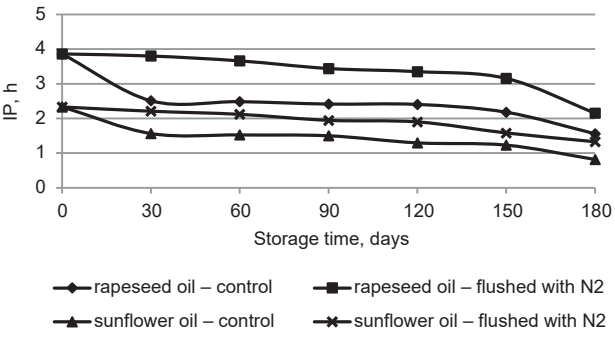

Fig. 4. Decrease of IP [h] during 180 days of storage in long-term test

light and temperature of 20°C), the PV value of flushing with nitrogen oil after 6 months of storage was 2.3 and 2.8 times lower, respectively, than in the control sample (Fig. 2). In the storage test, oil oxidation was not completely inhibited, but still slowly progressed owing the access of light (photosensibilized oxidation) (Choe and Min, 2006; Drozdowski, 2007).

In addition, an intensive increase was noted in the anisidine value (AnV) in both oils protected with nitrogen, during both tests (accelerated and long-term storage), which was likely due to intensive breakdown of the primary (PV at a stable level) to secondary products of oxidation. The flushing with nitrogen caused 2-fold deceleration of the increase of secondary oxidation product compared to the control samples, but still it did not cause complete reduction of these undesirable oxidative changes (Table 1, 2). Considering the oxidative stability of oils in the Rancimat test, it was found that in the accelerated test the IP changed insignificantly after 20 days of the test, whereas in the storage test with the access of light it was almost 2-fold shortened, however to a lesser extent in the oils packed with nitrogen (Fig. 3, 4).

Multivariate statistical analysis

Results obtained by PCA applied to correlation matrix of the analytical data set (AV, PV, AnV and IP), are depicted in Figure 5A and 6A. The first two principal components accounted for 92.32% (PC1 = 63.77% and PC2 = 28.55%, Schaal oven test) and 92.65% (PC1 = 62.89% and PC2 = 31.21%, long-term storage test) of the total variance. A significantly higher impact on the explanation of the variability was found in variables related to the oil oxidative status, namely PV (0.941;

Table 3. Pearson correlation coefficients between oil quality indices and IP of oil during Schaal oven test

Variables	AV	PV	AnV	Totox index	IP
AV	1				
PV	0.106	1			
AnV	–0.334	0.699	1		
Totox index	0.091	1.000	0.717	1	
IP	–0.581	–0.714	–0.324	–0.708	1

Table 4. Pearson correlation coefficients between oil quality indices and IP of oil during long-term storage test

Variables	AV	PV	AnV	Totox index	IP
AV	1				
PV	0.366	1			
AnV	–0.082	0.619	1		
Totox index	0.337	0.997	0.675	1	
IP	–0.866	–0.635	–0.184	–0.614	1

Table 5. Principal component analysis (PCA) factor loadings for the quality indices of the examined oils

Quality indices	Schaal oven test (63°C)		Long-term storage test (20°C)	
	PC1	PC2	PC1	PC2
AV	0.607	**0.757**	0.195	**0.748**
PV	**0.941**	–0.225	**0.975**	–0.078
AnV	0.603	–0.684	**0.736**	–0.570
Totox index	**0.941**	–0.274	**0.977**	–0.096
IP	**–0.828**	–0.510	**–0.811**	–0.498

Values in bold are loadings with an absolute value greater than 0.70.

0.954), Totox index (0.941; 0.977), and IP (–0.828; –0.811), than in variables related to the degree of hydrolysis of oils (AV: 0.757; 0.748) (Table 5). As can be seen from Table 3 and 4, it was found that, in both storage tests applied, there was a negative correlation between IP and oil quality indices studied (AV, PV, AnV and Totox index).

The PCA graph revealed that samples distribution on the bi-plot was primarily determined by the packaging atmosphere and clearly showed the differences between oils. When analysing results obtained from both storage tests (Schaal oven and long-term), it can be seen that on the PC plane, oil samples stored under nitrogen are placed on the left portion of the bi-plot, while control oil samples are located in the left zone of the plane (Fig. 5A, 6A, respectively).

Similarly to PCA, a dendrogram formed using the nearest neighbour method with Euclidean distances indicated similar samples grouping and clearly showed differences between oils stored under different conditions. When analysing dendrogram formed by oil samples subjected to accelerated Schaal oven test, two clusters were found (Fig. 5B). One consisting of control sunflower oil samples, while cluster 2 contains two distinct subclusters: first joins control rapeseed oil samples, second combines samples of fresh and stored under nitrogen rapeseed and sunflower oils. The samples of control sunflower oil was linked to the other cluster at large distance value which revealed the fastest rate of oxidative changes in this oils during accelerated stability test. Figure 6B presents the resulted dendrogram obtained from HCA of fresh and subjected to long-term storage oils, where 3 clusters can be identified. Cluster 1 contains control samples of sunflower and rapeseed oils with storage times longer than 60 days. Cluster 2 contains two distinct subclusters: first joins control rapeseed oil samples stored for 60 to 120 days, while seconds joins sunflower oil samples stored under nitrogen for 150 to 180 days. Cluster 3, formed by oils stored under nitrogen for no longer than 120 days and fresh oil samples, which indicates that these storage conditions were able to maintain oil quality parameters similar to those found in fresh cold-pressed oil samples.

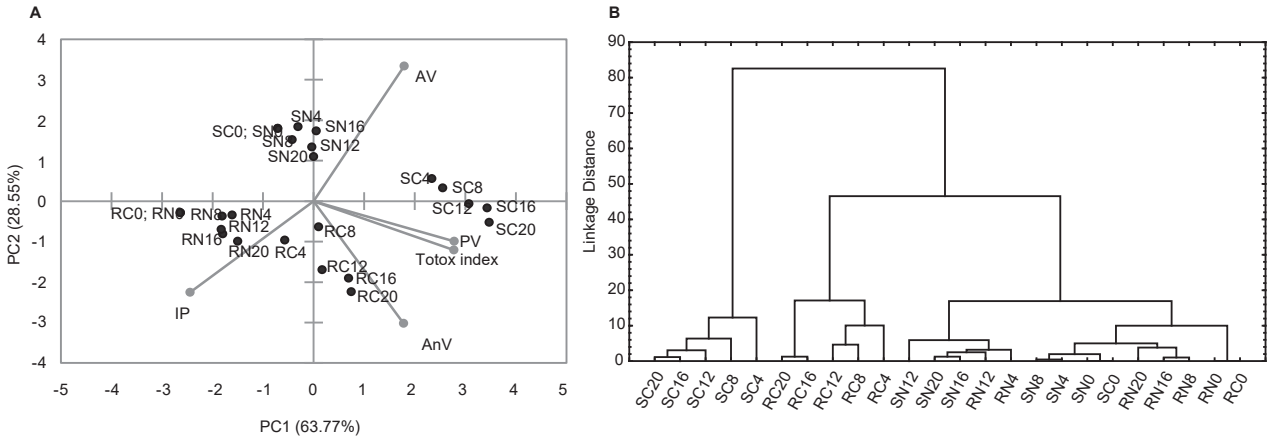

Fig. 5. PCA bi-plot (A) and HCA dendrogram (B) of oils subjected to Schaal oven test: R/S – oil (rapeseed/sunflower), C/N – nitrogen flushing (control/flushed with N_2), number – storage time (0, 4, 8, 12, 16, 20)

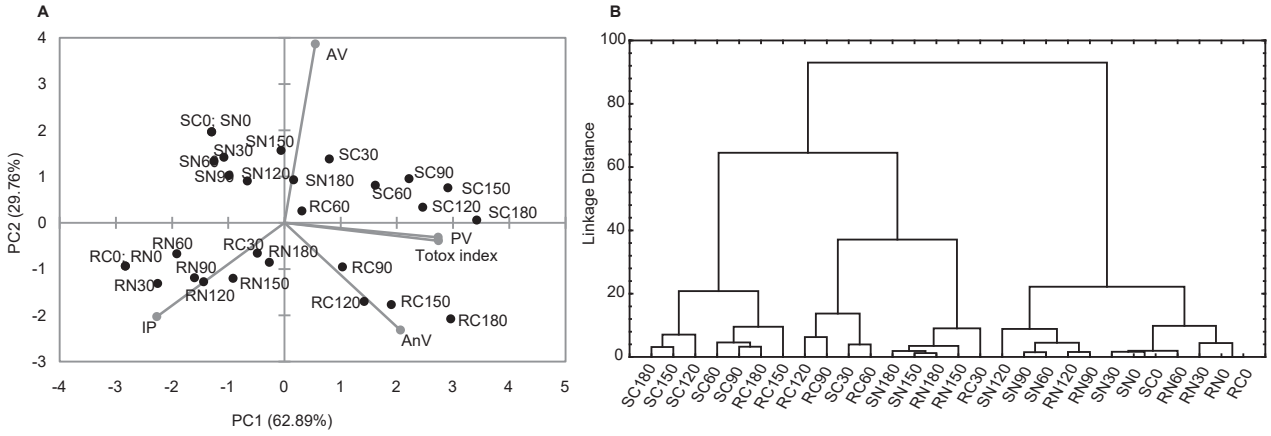

Fig. 6. PCA bi-plot and HCA dendrogram of oils subjected to long-term storage: R/S – oil (rapeseed/sunflower), C/N – nitrogen flushing (control/flushed with N_2), number – storage time (0, 30, 60, 90, 150, 180)

CONCLUSIONS

In this study it was demonstrated that the flushing of cold-pressed rapeseed and sunflower oils with nitrogen significantly decelerated oxidative processes (formation of peroxides), but did not eliminate all undesirable oxidative changes. A rapid increase in the content of secondary oxidation products was observed in both Schaal oven and long-term storage tests, after 30 and 60 days of storage, respectively. However, during that time, the peroxide value of flushing with nitrogen oils was 1.8 to 5.8-fold lower (Schaal oven test) and 4.4 to 5.4-fold lower (long-term storage test), compared to control samples. Shelf-life of cold-pressed oils flushed with nitrogen, stored at 20°C with the access of light, was elongated to 180 and 150 days, for rapeseed and sunflower oil, respectively. Oxidative stability of oils, determined via Rancimat tests, has been found to decline with prolonged storage, however, after 180 days of storage, the induction period of flushing with nitrogen oils was nearly 2-fold higher, compared to control oils.

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