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ORIGINAL PAPER

EFFECT OF GRAIN PRESERVATION ON THE CAROTENOID AND TOCOPHEROL CONTENT OF CEREAL GRAINS*

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Abstract

The aim of this study was to determine the effects of different wet grain preservation methods on the concentrations of β-carotene, xanthophyll, α-tocopherol (α-T), β-tocopherol (β-T), γ-tocopherol (γ-T) and δ-tocopherol (δ-T). Wet oat, triticale, barley and maize grain was dried at temp. of 60°C or 110°C and ensiled without additives or with 0.5% addition of propionic acid (+PA) or formic acid (+FA). Dried and ensiled maize grain had a higher content of β-carotene, xanthophyll, α-T, β-T, γ-T, δ-T, total tocopherols and vitamin E equivalent (Vit. EEq) than dried and ensiled oat, triticale and barley grain ($P \leq 0.01$). In dried cereal grain, the average losses of β-carotene, xanthophyll, α-T, β-T, γ-T, δ-T, total tocopherols and Vit. EEq were 57, 35, 84, 65, 67, 52, 74 and 81%, respectively, compared with their concentrations in wet grain ("0"). The content of xanthophyll, total tocopherols and Vit. EEq in grain decreased with an increase in drying temperature ($P \leq 0.01$). In ensiled cereal grain, the average losses of β-carotene, xanthophyll, α-T, β-T, γ-T, δ-T, total tocopherols and Vit. EEq were 52, 30, 83 50, 78, 63, 75 and 79%, respectively. Additives +PA and +FA affected only the β-T content ($P \leq 0.01$) of cereal silage. Grain drying resulted in greater losses of β-carotene, xanthophyll, α-T, β-T and Vit. EEq, whereas ensiling led to greater losses of γ-T, δ-T and total tocopherols. Dried triticale grain was characterized by greater losses of β-carotene, α-T, δ-T and total tocopherols, and ensiled oat grain was characterized by greater losses of α-T, γ-T, total tocopherol and Vit. EEq relative to the corresponding samples of dried and ensiled grain of the remaining cereal species.

Keywords: preservation, grain, carotenoids, tocopherols.

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INTRODUCTION

Among bioactive compounds, tocopherols and carotenoids play a particularly important biological role. They are the main lipophilic secondary metabolites with potent antioxidant activity, manifested by their ability to scavenge free radicals and control non-enzymatic lipid peroxidation during storage, seed germination and seedling growth (Maeda, DellaPenna 2007, Suriano et al. 2020). The tocopherol family includes four chemically distinct molecules: α-, β-, γ- and δ-tocopherol (α-T, β-T, γ-T and δ-T, respectively), of which α-T has the greatest biological activity (Panfili et al. 2008, Suriano et al. 2020). Carotenoids are classified into carotenes, such as α- and β-carotene, and xanthophylls (oxygenated derivatives of carotenes), among which lutein is most abundant, followed by zeaxanthin and β-cryptoxanthin (Adom et al. 2005, Suriano et al. 2020). β-carotene is the most effective vitamin A precursor among all carotenoids (Antoszkiewicz et al. 2019). Most of the tocopherols and carotenoids are located in the seed coat, the embryo and the aleurone layer of kernels. α-tocopherol dominates in the green parts of plants, whereas γ-T dominates in stems, roots, fruits, seeds, tubers, cotyledons, germinating seedlings and flowers (Haros et al. 2003, Horvath et al. 2006, Fardet et al. 2008, Asensi-Fabado, Munné-Bosch 2010). α-carotene and β-carotene are concentrated mainly in the germ, while lutein is equally distributed across the kernel (Panfili et al. 2004).

Plant-based feeds undergo changes during storage as a result of respiration, transpiration, and metabolic and microbial transformations which degrade carotenoids and tocopherols (Zieliński et al. 2001). In Central and Eastern Europe, wet grain of oats, triticale, barley and maize is often harvested under adverse weather conditions. Wet grain must be dried, which is an energy-consuming and expensive operation. Grain intended for sowing is dried at the temperature of the drying agent not exceeding 35-45°C, grain intended for consumption is dried at 50-60°C, and higher temperatures (110°C) are used to obtain products or semi-finished products with a considerably lower water content or those intended for further processing (Jayas, White 2003). Thermal processing of cereal grain improves nutrient digestibility, but it also affects the tocopherol content. In extruded oat, rye, barley and wheat grain, the average losses of α-T and β-T reach 80% and 55%, respectively (Zieliński et al. 2001). Ensiling is an alternative method for preserving wet feed, which also contributes to variations in the content of carotenoids and tocopherols due to fermentation and storage losses (Pieper et al. 2011).

The aim of this study was to determine the effects of different wet grain preservation methods on the concentrations of carotenoids and tocopherols. We hypothesized that the decrease in the content of important biologically active compounds caused by grain drying, the most popular preservation method, could be limited if alternative preservation methods, including ensiling, were used.

MATERIALS AND METHODS

Materials

The experimental materials comprised the grain of oats cv. Sławko, winter triticale cv. Marko, spring barley cv. Brenda, and Pioneer PR39K13 dent maize, harvested on 15, 22, 20 July and 21 October 2012, respectively, with the Bizon BS-2110 harvester. Samples of wet grain were collected in plastic bags with a capacity of 20 L directly from the trailer during the emptying of the harvester tank, and they were delivered to the laboratory four hours after harvest. Samples of wet grain ("0") were ground in a laboratory mill with cooling, to size <1 mm (Foss Knifetec 1095 Sample Mill) upon delivery, and their content of carotenoids and tocopherols was determined. Wet grain intended for ensiling was ground in a smooth roller mill, to size 1 - 4 mm (GM 325 FSC Lublin co. Poland), and ensiled under laboratory conditions, in tightly closed twist glass containers with a capacity of 1 dm^3, without additives ("00") or with the 0.5% addition of propionic acid, + PA (99%, Sigma-Aldrich) or 0.5% of formic acid, + FA (88%, Sigma-Aldrich) – Table 1. Propionic acid and formic acid, in the amount of around 100 cm^3, were sprayed with through a dispersing nozzle of a hand-held garden sprayer of a capacity of 500 cm^3 over a layer of crushed grain, which was then mixed by hand. Silage was stored out of sunlight, under laboratory conditions, at room temp. of around 20°C for six weeks. The remaining samples of wet grain (approx. 800 g each) were placed in aluminum cuvettes (175 x 280 x 40 mm) in layers 20 mm thick, and were dried simultaneously at a temp. of 60°C or 110°C, in two laboratory dryers (BINDER FED) with forced air circulation, for 18 h (Table 1).

Table 1

Experimental design

Specification	Oats	Triticale	Barley	Maize	Σ
	n	*n*	*n*	*n*	
"0"	8	8	8	16	40
60°C	8	8	8	16	40
110°C	8	8	8	16	40
Σ	24	24	24	48	120
"00"	8	8	8	16	40
+PA	8	8	8	16	40
+FA	8	8	8	16	40
Σ	24	24	24	48	120

"0" – freshly harvested cereal grain; 60°C – grain dried at 60°C; 110°C – grain dried at 110°C; "00" – grain ensiled without silage additives; + PA – grain ensiled with 0.5% addition of propionic acid; + FA – grain ensiled with 0.5% addition of formic acid

Chemical analyses and methods

Chemical composition of grain

The content of dry matter, crude protein, crude fiber and crude fat in grain samples was determined according to the official methods of analysis of the Association of Official Analytical Chemists (AOAC) (2007). Dry matter was determined by the gravimetric method after drying at 105°C. Crude fiber was determined by the enzymatic-gravimetric method, crude protein was determined by the Kjeldahl method, and crude fat was determined by the *Soxhlet extraction* method.

Determination of the content of fermentation products in silage

The concentrations of lactic acid and volatile fatty acids were determined as described by Kostulak-Zielińska, Potkański (2001). Silage samples were homogenized with a Bosch blender in deionized water at a 5:1 ratio, and were filtered through polyamide gauze. The filtrate was passed through a soft filter, deproteinized with 24% solution of metaphosphoric acid, and centrifuged (13000 x g). The supernatant was analyzed to determine the concentrations of ethanol, lactic acid and volatile fatty acids (formic acid, acetic acid, propionic acid, butyric acid, valeric acid). Volatile fatty acids and ethanol were separated and determined by gas chromatography with a Varian CP-8410 autosampler coupled with a flame-ionization detector (FID) and a CP-FFAP capillary column (length – 25 m, inner diameter – 0.53 mm, film thickness – 1.0 μm), sample size – 1 μL, detector temp. – 260°C, injector temp. – 200°C, column temperature – 90°C to 200°C, carrier gas – helium (flowrate – 5.0 mL min^{-1}). Lactic acid content was determined by high performance liquid chromatography (HPLC, Shimadzu, Kioto, Japan) with isocratic flow. Separation was carried out using a Varian Metacarb 67H column (Palo Alto, USA), mobile phase – 0.002 mol L^{-1} solution of sulfuric acid in deionized water, flow rate – 1 cm^3 min^{-1}, UV detector, 210 nm. External fatty acid standards were supplied by Supelco (Bellefonte, USA), and the lactic acid standard – by Fluka (Buchs, Switzerland). Other determinations included pH (HI 8314N pH – meter, Hanna Instruments) and N-NH_3 (Conway method).

Determination of the content of β-carotene, xanthophyll and tocopherols

Samples of freshly harvested grain and dried grain were ground in a laboratory mill with cooling (Foss Knifetec 1095 Sample Mill), silage samples were ground with a Bosch blender, and all samples were homogenized in an ice-water bath (Ultra Turrax homogenizer, Janke & Kunkel IKA – Labortechnik). The content of β-carotene, xanthophyll and tocopherols was determined under limited exposure to sunlight. Ground samples were weighed (10 g), extracted with 50 cm^3 of the petroleum ether:acetone mixture (1:1) at room temperature, left in the dark for 18 h, saponified with ethanolic 10% KOH solution (2 h in the dark, under nitrogen atmosphere), extracted twice

with 20 cm^3 of ethanol (96%), and then repeatedly with petroleum ether. The extracts, combined in a separating funnel, were washed successively with 10% aqueous NaCl solution and several times with deionized water, the eluates were dehydrated with anhydrous sodium sulfate, and evaporated to dryness (40°C) on a rotary evaporator (Janke & Kunkel IKA – Labortechnik). To determine the content of β-carotene, xanthophyll and tocopherols, the residue was dissolved in 5 cm^3 of hexane. The content of β-carotene and xanthophyll was determined by RP-HPLC (Shimadzu) on a Gemini 5 µ C18 column (Phenomenex), 110Å, 250x4 mm, mobile phase: acetonitrile:methanol:dichloromethane (750:200:50), flow rate: 1 cm^3 min^{-1}, UV-vis detector, 450 nm, external standards: β-carotene type I, synthetic, xanthophyll lutein; β-carotene-3,3'-diol (Sigma-Aldrich) – Manz (1986), de Quirós, Costa (2006). The content of tocopherols was determined by RP-HPLC (Shimadzu), Nucleosil C_{18} column, mobile phase: methanol: H_2O (95:5), flow rate: 1 cm^3 min^{-1}, RF detector Ex 293 and Em 326, external standards: (±)-α-T (DL-all-rac α-T), β-T, (+)-γ-T, (+)-δ-T (Sigma-Aldrich) – PN-EN-ISO 6867:2002. The value of Vit. EEq was calculated as described by Eittenmiller et al. (1998).

Statistical analysis

The results were analyzed statistically by two-way ANOVA. A model was constructed to determine the effect of drying temperature or silage additive on the content of carotenoids and tocopherols in the analyzed cereal grain. The significance of differences between means was verified by the Duncan's test. The interactions between cereal species and drying temperature or silage additive influencing the content of basic nutrients, fermentation products, carotenoids and tocopherols in dried and ensiled grain were determined. All calculations were performed using Statistica version 13.3 software.

RESULTS

Chemical composition of wet, dried and ensiled cereal grain, and the content of fermentation products in silage

Wet grain of oats, triticale and barley had similar dry matter content (approx. 756.8 g kg^1), which was higher than in maize grain ($P \leq 0.01$) – Table 2. Barley grain had the highest crude protein content ($P \leq 0.01$), maize grain had the highest content of crude fat and N-free extracts ($P \leq 0.01$), and oat grain had the highest content of crude fiber ($P \leq 0.01$) – Table 2. The higher value of N-free extracts / N x 6.25 could be indicative of a greater fermentation potential of triticale and maize grain, compared with the grain of the remaining cereal species ($P \leq 0.01$) – Table 2. Wet maize grain was characterized by a higher content of carotenoids (β-carotene content was more than 4 times higher, and xanthophyll was more than 10 times higher) and tocopherols

Table 2

Chemical composition (g kg^{-1} DM) and the content of β-carotene, xanthophyll and tocopherols (mg kg^{-1} DM) in freshly-harvested cereal grain („0").

Specification	Species					
	oats	triticale	barley	maize	SEM	$\bar{x}$
Dry matter (g kg^{-1})	762.0^{A}	750.2^{A}	758.1^{A}	651.2^{B}	7.36	730.4
Crude protein	119.9^{C}	125.6^{B}	138.2^{A}	93.6^{D}	1.31	119.3
Crude fat	40.0^{B}	14.3^{D}	17.9^{C}	46.8^{A}	0.55	29.8
Crude fiber	103.9^{A}	39.1^{BC}	49.9^{B}	28.8^{C}	2.68	55.4
N-free extracts	7116^{C}	801.3^{Ab}	771.7^{B}	815.3^{Aa}	3.62	774.9
N-free extracts / N x 6.25	5.9^{C}	6.4^{B}	5.6^{D}	8.7^{A}	0.22	6.7
β-carotene	0.73^{B}	0.83^{B}	0.98^{B}	3.95^{A}	0.08	1.62
Xanthophyll	2.01^{B}	2.14^{B}	2.84^{B}	27.16^{A}	0.95	8.54
α-tocopherol	5.04^{B}	5.15^{B}	5.24^{B}	7.63^{A}	0.19	5.77
β-tocopherol	1.75^{C}	$1,89^{BCb}$	2.20^{BCa}	3.49^{A}	0.06	2.33
γ-tocopherol	2.92^{B}	3.08^{B}	2.37^{C}	3.65^{A}	0.29	3.01
δ-tocopherol	0.96^{B}	2.23^{A}	0.17^{C}	1.78^{A}	0.15	1.29
Total tocopherols	10.67^{BCb}	12.34^{Ba}	9.98^{C}	16.55^{A}	0.45	12.39
Vit. EEq	6.13^{Bb}	6.24^{B}	6.37^{Ba}	9.41^{A}	0.17	7.04

Values not sharing the same superscript within a row are significantly different at $p \leq 0.05$ (*a*, *b*) or $p \leq 0.01$ (*A*, *B*, *C*).

(on average by about 34% more) compared with the grain of the remaining cereal species (Table 2). Wet grain of oats, triticale and barley was characterized by a similar content of biologically active compounds. However, it is worth pointing to a slightly higher content of β-carotene, xanthophyll, α- and β-tocopherol and a slightly higher value of vit. EEq in barley grain than in oat and triticale grain (Table 2).

The content of crude protein ($P \leq 0.01$), crude fat ($P \leq 0.01$), crude fiber ($P \leq 0.01$) and N-free extracts ($P \leq 0.01$) in dried grain was affected by cereal species (Table 3). Drying temperature (60°C, 110°C) affected dry matter content ($P \leq 0.01$), but it had no significant effect on the content of basic nutrients in grain samples. An interaction was found between cereal species and drying temp. for the content of basic nutrients in grain ($P \leq 0.01$) – Table 3. The content of basic nutrients (Table 3), N-NH_3, lactic acid, acetic acid and butyric acid in silage as well as silage pH (Table 4) were affected ($P \leq 0.01$) by cereal species. Additives +PA and +FA had no significant effect on the proximate chemical composition of silage (Table 3), but they affected ($P \leq 0.01$) the content of N-NH_3, formic acid, acetic acid, propionic acid and valeric acid in silage, relative to grain ensiled without additives ("00") – Table 4. An interaction was noted between cereal species and silage additives (+PA, +FA) for the content of basic nutrients ($P \leq 0.01$) (excluding dry

Table 3

Chemical composition (g kg^{-1} DM) of dried and ensiled cereal grain, including the interactions between cereal species and drying temperature, and between cereal species and silage additives

Specification	Dry matter (g kg^{-1})	Crude protein	Crude fat	Crude fiber	N-free extracts
Dried grain					
Oats	924.1	124.5^{B}	33.1^{B}	110.1^{A}	708.3^{C}
Triticale	944.4	120.1^{B}	15.4^{D}	34.0^{B}	810.1^{A}
Barley	936.8	128.7^{A}	22.5^{C}	30.8^{C}	785.7^{B}
Maize	931.7	91.6^{C}	50.3^{A}	33.9^{B}	808.0^{A}
Drying (D)					
"0"	730.4^{C}	119.4	29.8	55.2	775.3
60°C	911.2^{B}	116.5	32.5	51.8	778.2
110°C	957.2^{A}	116.0	33.1	52.6	777.9
Effect, *P*-value					
Grain (G)	0.004	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Drying (D)	≤ 0.01	0.631	0.559	0.898	0.956
Interaction (G×D)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
SEM	8.579	1.564	1.337	3.317	4.253
Ensiled grain					
Oats	725.2^{A}	127.8^{B}	39.9^{B}	91.4^{A}	716.1^{D}
Triticale	730.8^{A}	129.0^{B}	14.5^{C}	33.5^{C}	803.3^{B}
Barley	678.7^{Ba}	151.9^{A}	15.9^{C}	52.1^{C}	755.1^{C}
Maize	648.4^{Bb}	92.6^{C}	49.6^{A}	25.2^{D}	817.1^{A}
Additives (A)					
"00"	691.2	126.7	29.4	51.3	771.2
+ PA	700.5	123.9	29.4	51.0	774.5
+FA	695.3	125.4	31.2	49.4	772.9
Effect, *P*-value					
Grain (G)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Additives (A)	0.811	0.877	0.873	0.950	0.951
Interaction (G×A)	0.974	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
SEM	5.834	2.193	1.594	2.628	4.178

Values not sharing the same superscript within a row are significantly different at $p \leq 0.05$ (*a*, *b*) or $p \leq 0.01$ (*A*, *B*, *C*).

matter) – Table 3 and fermentation products ($P \leq 0.01$) – Table 4 in ensiled cereal grain.

Table 4

The pH value and content of fermentation products (g kg^{-1} DM) in cereal silage, including the interactions between cereal species and silage additives

Specification	pH	$N\text{-}NH_3$ (g kg^{-1} N)	Lactic acid	Formic acid	Acetic acid	Propionic acid	Ethanol	Butyric acid	Valeric acid
Ensiled grain									
Oats	4.8^{B}	2.6^{A}	14.4^{B}	7.2	8.9^{B}	12.5	0.9^{Aa}	0.3^{B}	1.9
Triticale	5.3^{A}	2.5^{A}	8.7^{Ca}	7.3	12.9^{A}	10.7	0.3^{b}	0.2^{B}	1.6
Barley	4.8^{B}	1.3^{B}	6.5^{Cb}	7.9	5.13	10.7	0.01^{B}	0.6^{B}	0.9
Maize	3.8^{C}	2.8^{A}	18.5^{A}	7.5	8.2^{B}	9.8	0.5	1.5^{A}	2.6
Additives (A)									
"00"	5.0^{A}	2.8^{A}	11.9	0.2^{B}	10.7^{Aa}	1.7^{B}	0.4	0.8	3.4^{A}
+ PA	4.6	12.0^{B}	14.2^{A}	0.1^{B}	8.8^{b}	30.0^{A}	0.6	0.5	0.9^{B}
+FA	4.4^{B}	2.1^{B}	10.0^{B}	22.1^{A}	6.9^{Ba}	1.0^{B}	0.3	0.7	0.9^{B}
Effect, *P*-value									
Grain (G)	≤ 0.01	≤ 0.01	≤ 0.01	0.997	≤ 0.01	0.925	0.013	≤ 0.01	0.155
Additives (A)	0.022	≤ 0.01	0.012	≤ 0.01	≤ 0.01	≤ 0.01	0.350	0.041	≤ 0.01
Interaction (G×A)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
SEM	0.078	0.088	0.587	1.063	0.363	1.409	0.098	0.056	0.269

Values not sharing the same superscript within a row are significantly different at $p \leq 0.05$ (*a*, *b*) or $p \leq 0.01$ (*A*, *B*, *C*).

The content of carotenoids and tocopherols in wet, dried and ensiled cereal grain

Wet maize grain ("0") had a higher content of β-carotene, xanthophyll, tocopherols, total tocopherols and Vit. EEq ($P \leq 0.01$) than wet grain of the remaining cereal species (Table 2). Cereal species affected the concentrations of carotenoids ($P \leq 0.01$) and tocopherols ($P \leq 0.01$) in dried grain (Table 5). Dried maize grain had a higher ($P \leq 0.01$) content of β-carotene, xanthophyll, α-T, β-T, γ-T, δ-T, total tocopherols and Vit. EEq than the dried grain of other cereals (Table 5). It should be noted that the differences of β-carotene, α-T, γ-T, δ-T and total tocopherols in dried triticale grain reached 76, 93, 80, 84 and 85%, respectively, compared with the content of these components in wet grain ("0") – Table 2, and they were greater than in the dried grain of the remaining cereal species. The content of xanthophyll, tocopherols, total tocopherols and Vit. EEq in grain decreased with increasing drying temp. ($P \leq 0.01$) – Table 5. The content of α-T, β-T, γ-T, δ-T, total tocopherols and Vit. EEq was lower ($P \leq 0.01$) in grain dried at 110°C than in grain dried at 60°C by 27, 47, 37, 45, 37 and 35%, respectively (Table 5). A comparison

Table 5

The content of β-carotene, xanthophyll, tocopherols, total-tocopherols and vitamin E equivalent (mg kg^{-1} DM) in dried and ensiled cereal grain, including the interactions between cereal species and drying temperature, and between cereal species and silage additives

Specification	β-carotene	Xanthophyll	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols	Vit. E Eq
Dried grain								
Oats	0.43^{B}	1.21^{C}	0.43^{B}	0.36^{Bb}	0.57^{B}	0.41^{B}	1.77^{BC}	0.64^{C}
Triticale	0.20^{C}	1.54^{B}	0.38^{C}	0.54^{B}	0.63^{B}	0.35^{C}	1.90^{B}	0.66^{C}
Barley	0.30^{B}	1.71^{B}	0.59^{B}	0.46^{Ba}	0.44^{C}	0.12^{D}	1.61^{C}	$0,82^{B}$
Maize	2.32^{A}	19.03^{A}	2.88^{A}	2.40^{A}	2.66^{A}	1.14^{A}	9.08^{A}	4.12^{A}
Drying (D)								
"0"	1.62^{A}	8.54^{Aa}	5.77^{A}	2.33^{A}	3.01^{A}	1.29^{A}	12.39^{A}	7.02^{A}
60°C	0.85^{B}	6.80^{Aba}	1.24^{B}	1.23^{B}	1.32^{B}	0.65^{B}	4.38^{B}	1.93^{B}
110°C	0.77^{B}	4.94^{Bb}	0.90^{C}	0.65^{C}	0.83^{C}	0.36^{C}	2.74^{C}	1.25^{C}
Effect, *P*-value								
Grain (G)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Drying (D)	0.003	0.285	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Interaction (G×D)	≤ 0.01	≤ 0.01	0.529	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
SEM	0.116	0.923	0.255	0.115	0.129	0.074	0.534	0.307
Ensiled grain								
Oats	0.21^{C}	1.40^{C}	0.43^{C}	0.84^{B}	0.54^{B}	0.16^{B}	1.97^{C}	0.82^{Bb}
Triticale	0.43^{B}	1.63^{BCb}	0.53^{BCb}	1.15^{A}	0.62^{B}	0.15^{B}	2.46^{B}	1.05^{B}
Barley	0.27^{C}	2.16^{Ba}	0.72^{Ba}	1.22^{A}	0.48^{B}	0.13^{B}	2.55^{B}	1.26^{Ba}
Maize	3.37^{A}	15.57^{A}	2.59^{A}	1.33^{A}	1.18^{A}	0.88^{A}	5.99^{A}	3.25^{A}
Additives (A)								
"00"	1.15	5.25	1.29	0.99^{B}	0.75	0.34	3.37	1.76
+ PA	1.09	5.42	0.93	1.07^{A}	0.77	0.32	3.08	1.44
+FA	0.97	4.89	0.99	1.34^{A}	0.61	0.33	3.27	1.59
Effect, *P*-value								
Grain (G)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Additives (A)	0.860	0.940	0.256	≤ 0.01	0.297	0.982	0.790	0,523
Interaction (G×A)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	0,172
SEM	0.096	0.038	0.044	0.034	0.170	0.138	0.621	0.120

Values not sharing the same superscript within a row are significantly different at $p \leq 0.05$ (*a*, *b*) or $p \leq 0.01$ (*A*, *B*, *C*).

of the concentrations of carotenoids and tocopherols in unprocessed cereal grain (Table 2) and in dried grain revealed the following average differences: β-carotene – 57%, xanthophyll – 35%, α-T – 84%, β-T – 65%, γ-T – 67%, δ-T – 52%, total tocopherols – 74%, and Vit. EEq - 81%. No interaction was found between cereal species and drying temperature for α-T content (Table 5).

This study confirmed that cereal species affected the content of carotenoids ($P \leq 0.01$) and tocopherols ($P \leq 0.01$) in ensiled grain. Ensiled maize grain had a higher ($P \leq 0.01$) content of β-carotene, xanthophyll, α-T, β-T, γ-T, δ-T, total tocopherols and Vit. EEq than the ensiled grain of oats, triticale and barley (Table 5). Ensiled oat grain had the lowest content of β-carotene, xanthophyll, α-T, total tocopherols ($P \leq 0.01$) and Vit. EEq ($P \leq 0.01$, $P \leq 0.05$), compared with the ensiled grain of the remaining cereal species (Table 5). Ensiling contributed to the difference of carotenoids and tocopherols relative to their content in fresh grain ("0"). The greatest differences of β-carotene were found in ensiled barley grain (72%), of xanthophyll – in ensiled maize grain (43%), of α-T, γ-T, total tocopherols and Vit. EEq – in ensiled oat grain (91, 82, 82 and 87%, respectively), compared with the content of these components in the raw material ("0"). Silage additives +FA and +PA increased ($P \leq 0.01$) the β-T content of ensiled grain (Table 5).

DISCUSSION

The content of crude protein, crude fat and N-free extracts (Tables 2 and 3) in unprocessed ("0"), dried (60°C or 110°C) and ensiled grain ("00", + PA or + FA), determined in the present study, was similar to the chemical composition of oat, triticale, barley and maize grain reported by Maeda et al. (1997), Hackl et al. (2010) and Pieper et al. (2011). The β-carotene content of fresh maize grain ("0") remained within the range of 0.01-11 mg kg^{-1} DM reported by Ortiz-Monasterio et al. (2007), Fardet et al. (2008), Menkir et al. (2008) and Hossain, Jayadeep (2018), and was it higher ($P \leq 0.01$) than the β-carotene content of oat, triticale and barley grain ("0") – Table 2. The grain of oats, triticale and barley used in the current study contained 0.84 mg kg^{-1} β-carotene on a dry matter basis (Table 2). This value is somewhat higher than those determined by Humphries et al. (2004) and Fardet et al. (2008) (0.032-0.7 mg kg^{-1} DM). The xanthophyll content of the analyzed cereal grain ("0") was many times higher than β-carotene content (Table 2). However, the xanthophyll content of cereal grain noted in the present study is consistent with the values reported by Humphries et al. (2004), Fardet et al. (2008) and Sajilata et al. (2008) in whose studies xanthophyll concentrations ranged from 11 to 30 mg kg^{-1} DM in maize grain, and from 0.3 to 13 mg kg^{-1} DM in wheat and triticale grain. The above authors observed high variability in xanthophyll levels in the grain of different varieties of the same cereal species.

Drying reduced the content of β-carotene and xanthophylls in the analyzed cereal grain, relative to their concentrations in freshly-harvested grain ("0") – Tables 2 and 5. Dried oat and maize grain was characterized by lower β-carotene differences (41%), and triticale and maize grain – by lower xanthophyll differences (28% and 30%, respectively) than the grain of the remaining cereal species (Table 5). According to SCHIEBER, CARLE (2005), thermally processed products contain less carotenes in the trans-isomeric configuration and more cis-isomers whose pro-vitamin A activity, bioavailability and antioxidant capacity are lower, which alters the biological properties of β-carotene.

Grain preserved with additive +PA had higher ($P \leq 0.01$) lactic acid content than the remaining silages ("00", +FA) – Table 4. NOZIÈRE et al. (2006) reported a beneficial effect of a higher content of lactic acid on the stability of carotenoids in green forage silage, which was not, however, fully confirmed by the present findings. The concentration of lactic acid was highest ($P \leq 0.01$) in ensiled maize grain (Table 4), similarly to the content of carotenoids and tocopherols (Table 5). Ensiled oat grain had a higher concentration of lactic acid ($P \leq 0.01$) – Table 4, but lower concentrations of carotenoids and tocopherols ($P \leq 0.01$) than the other evaluated silages (Table 5).

In samples of freshly harvested maize grain ("0"), the content of all forms of tocopherols, total tocopherols and Vit. EEq was higher ($P \leq 0.01$) than in the grain of the remaining cereal species (Table 2). The α-T content of maize grain was similar to that reported by HIDIROGLOU et al. (1992) and PANFILI et al. (2008) – 6-7 mg kg^{-1} DM, and lower than that noted by HORVATH et al. (2006) – 18-23 mg kg^{-1} DM. TIWARI et CUMMINS (2009) reported significant variation in the content of α-T in maize grain (1.4-32.5 mg kg^{-1}). In samples of freshly harvested oat, triticale and barley grain ("0"), the average content of α-T and total tocopherols (5.1 and 11.0 mg kg^{-1} DM, respectively) – Table 2 was lower than the values of 11 mg kg^{-1} DM α-T and 21 mg kg^{-1} DM total tocopherols determined in oat and barley grain by CAVALLERO et al. (2004), FARDET et al. (2008) and PANFILI et al. (2008). In freshly-harvested cereal grain ("0"), α-T and γ-T had a higher share of the total tocopherols (47% and 24%, respectively) than the other forms of tocopherol (β-T – 19%, δ-T – 10%) –Table 2. Similar results were reported by CAVALLERO et al. (2004), FALK et al. (2004), HORVATH et al. (2006) and TSOCHATZIS et al. (2012) who noted a predominance of α-T, different concentrations of β-T and γ-T, and the absence or trace amounts of δ-T in cereal grains, seeds and fruits. According to the cited authors, cereal grain is a moderate source of tocopherols.

As a result of drying, the content of tocopherols decreased manyfold (Table 5) relative to freshly harvested grain ("0") – Table 2. HIDIROGLOU et al. (1992) reported an over two-fold higher content of vitamin E in dried maize compared with non-dried grain (9 vs. 20 mg kg^{-1}DM.). In cereal grain dried at 60°C and 110°C, the loss of α-T reached 79% and 84%, respectively, and it was higher than the loss of the other forms of tocopherol. In a study by

ZIELIŃSKI et al. (2001), the loss of α-T (73%) exceeded the losses of β-T (63%) and δ-T (45%) in cereal grain extruded at different temp. (200, 160 and 120°C). Grain drying at 60°C and 110° C reduced the content of total tocopherols (by 65% and 78%, respectively) and Vit. EEq (by 73% and 82%, respectively), compared with non-dried grain. The lowest concentration of total tocopherols was determined in dried barley grain ($P \leq 0.01$), which corroborates the findings of ZIELIŃSKI et al. (2001) who noted a lower concentration of total tocopherols in extruded barley grain than in the extruded grain of wheat, rye and oats.

In grain ensiled without silage additives ("00"), the content of α-T, total tocopherols and Vit. EEq was higher than in silage with the addition of acids, but the noted differences were not statistically significant (Table 5). Grain ensiled with additive +PA contained less α-T, total tocopherols and Vit. EEq, but no significant differences were found (Table 5). According to HIDIROGLOU et al. (1992), the use of +PA in the amount of 1% of the weight of the preserved grain leads to tocopherol losses. In the present study, the losses of β-carotene, xanthophyll, β-T and Vit. EEq were higher in dried than in ensiled grain – Table 5 (57% vs. 52%, 35% vs. 30%, 65% vs. 50%, 81% vs. 79%, respectively), whereas the losses of γ-T and δ-T were higher in ensiled than in dried grain (78% vs. 65% and 63% vs. 52%, respectively). The losses of α-T (approx. 84%) and total tocopherols (approx. 75%) were similar regardless of the preservation method. Greater losses of β-carotene, α-T, δ-T and total tocopherols were determined in dried triticale grain, and greater losses of α-T, γ-T, total tocopherols and Vit. EEq were noted in ensiled oat grain than in samples of dried or ensiled grain of the remaining cereal species.

CONCLUSIONS

1. Wet, dried and ensiled maize grain was a richer source of carotenoids and tocopherols than wet and preserved grain of oats, triticale and barley.

2. Grain drying reduced the content of carotenoids, β-T and Vit. EEq, whereas ensiling contributed to a decrease in the content of γ-T and δ-T, compared with their concentrations in wet grain.

3. During grain preservation by drying and ensiling, β-carotene was less stable than xanthophyll, and α-T and γ-T were the least stable tocopherols.

4. Dried and ensiled oat and triticale grain was characterized by higher differences of α-T, total tocopherols and Vit. EEq than the grain of the other cereals.

5. The use of silage additives did not increase the content of important biologically active compounds such as β-carotene and α-T in ensiled cereal grain.

6. In the context of livestock feeding, ensiling seems to be the most effective method of grain preservation.

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Conflicts of interest

There are no conflicts of interests to declare.

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