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Differences in content and composition of free lipids and carotenoids in flour of spring and winter wheat cultivated in Poland

Iwona Konopka *, Sylwester Czaplicki, Daniela Rotkiewicz

Chair of Food Plant Chemistry and Processing, University of Warmia and Mazury in Olsztyn, Pl. Cieszyński 1, 10-957, Olsztyn, Poland

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Abstract

The work presents the content and composition of free lipids and carotenoids in spring and winter classes of wheat flour. It discusses genetical and physiological aspects of their synthesis and accumulation in wheat kernels and also indicates how methodological differences explain differences in results presented in the literature. It has been reported that spring wheat flours are richer in free lipids, especially in the non-polar fraction. The content of glycolipids ranged from 134 to 215 mg/100 g flour and was more stable within the winter wheat class. The percentages of the two main fractions, namely DGDG and MGDG, were similar in both wheat classes and reached ca. 77%. Phospholipids constituted the smallest fraction of the flour free lipids in both wheat classes; however, spring wheat flours were richer in these compounds, which is likely associated with a greater content of spherosomes in the endosperm of this wheat class. The free lipids of spring wheat flour contained more oleic and slightly less linoleic and linolenic acids. Spring wheat flour was also richer in carotenoids, although there were varieties in both classes that deviated from this. The main carotenoid was lutein, whose total percentage in the form of different isomers ranged from 71.3% to 83.3% and was slightly lower for spring wheat flour. Lutein, in the form of a *trans*-isomer, constituted about 62% and 70% of all carotenoids in spring and winter wheat flours, respectively.

Keywords: Carotenoids; Free lipids; Glycolipids; Wheat flour

1. Introduction

It is well known that the baking value of wheat flour depends mainly on the content and composition of proteins, but can be significantly modified by flour lipids (reviewed by Chung & Ohm, 1997). According to Marion and Clark (1995), the functionality of wheat lipids results from: (1) participation in redox processes, involving lipooxygenase-catalysed oxidation of polyunsaturated fatty acids, which has an effect on the rearrangement of disulphide bonds of gluten proteins, (2) involvement of lipids and lipid-protein complexes in the formation and stabilisation of air/water (foam) and oil/water (emulsion) interfaces, during dough mixing, proofing and baking. Among flour lipids, the so-called 'free fraction' that can be extracted by non-polar solvents is of great interest. The content of this fraction is also considerably more changeable than that of bound lipids, as was reported by Ruibal-Mendieta, Delacroix, and Meurens (2002) and Bekes, Zawistowska, Zillman, and Bushuk (1986), for winter and spring wheat, respectively.

Most studies into free lipids have concerned wheat cultivated in the US (Chung, Pomeranz, & Finney, 1982; Ohm & Chung, 1999, 2000, 2002), Canada (Bekes et al., 1986), Australia (Panozzo, Hannah, O'Brien, & Bekes, 1993), UK (Bell, Daniels, & Fearn, 1987), Hungary (Karpati, Bekes, Lasztity, & Oersi, 1990) and Greece (Matsoukas & Morrison, 1991). It was shown

* Corresponding author. Fax: +4889 523 34 66.
E-mail address: iwona.konopka@uwm.edu.pl (I. Konopka).

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that wheat lipids are quite a complex family of compounds with varied and often opposite effects on flour baking value. These papers, and particularly, the long-term studies carried out by Chung et al. (1982) and co-authors, provided evidence that free glycolipids, especially digalactosyldiglicerides (DGDG) play a key role in the improvement of flour baking value.

Although the biosynthetic mechanisms of plant lipids are quite well understood, their regulation is not (Moreau et al., 1998). Morrison, Law, Wylie, Coventry, and Seekings (1989) discovered that the free polar lipid level is controlled partly, by a gene allelic to hardness Ha locus on the short arm of chromosome 5D or closely linked to it. They also reported that variation in free lipids cannot be due to this gene alone and is probably modified by gene/genes on the long arm of this chromosome, proximal to *Vrn3* (the gene for spring and winter wheat habit).

The crude lipid fraction of flour also includes accompanying compounds of similar polarity in which carotenoids are of special nutritional importance. The main carotenoids of wheat are xanthophylls, predominantly lutein (Heinonen, Olliainen, Linkola, Varo, & Koivistoinen, 1989; Kaneko, Nagamine, & Yamada, 1995; Lepage & Sims, 1968). The second wheat grain carotenoid is zeaxanthin (Humphries & Khachik, 2003). The ratio of lutein to zeaxanthin is established at ca. 9.3 and 2.5 in mature and green-harvestaed wheat kernels, respectively (Humphries & Khachik, 2003). The same authors also stated that green-harvested wheat is much richer in carotenoids (about 1100 ng/g of grain in comparison to 35–250 ng/g of mature grain) and that both carotenoids appear predominantly in the form of *trans*-isomers.

The main classification of wheat is based on its growing habit and divides wheat varieties into winter and spring market classes. This diversity is caused by the vernalization genes *Vrn1* and *Vrn3* on wheat chromosomes 5AL and 5DL, respectively (Nelson et al., 1995). The objective of this study was to elucidate whether wheat growing habit (spring/winter) is connected with the specific accumulation of free lipids and carotenoids in grain. The latter is of especial significance for human health because lutein and zeaxanthin are known to prevent age-related macular degradation (see review by Snodderly, 1995). Their concentration in human macula can be manipulated by dietary intake (Bone, Landrum, Dixon, Chen, & Llerena, 2000), thus indicating that wheat class or varieties richer in these compounds are worth considering.

2. Materials and methods

2.1. Material

Eleven Polish wheat varieties (five spring and six winter wheats) cultivated in the same Crop Cultivation Station in north-eastern Poland, in 2003, were the subject of the study. Before analyses, the grain was sieve-separated and, for further investigations, only dominant fractions with widths larger than 2.8 mm were taken. Dry grain (approximately 12% of moisture) was stored in linen bags at 9 °C and a relative humidity of 70% and successively milled into extraction flour.

The extraction flours were obtained by milling the grain, tempered to 14% moisture content, in a

Table 1
Basic characteristics of used flours

	Variety	Flour yield (%)	Protein (%, d.b.)	Zeleny test (cm^3)	FWA (%)	Amylograph viscosity (BU)
Spring varieties	Zebra	71.97	13.4	52	59.74	880
	Jasna	68.47	13.7	50	61.11	950
	Olimpia	72.18	12.8	47	59.87	800
	Koksa	70.60	13.6	52	61.37	940
	Opatka	69.60	12.7	50	59.07	760
	$\bar{x}$	**70.56**[a]	**13.2**[a]	**50**[a]	**60.23**[a]	**866**[a]
	$\hat{s}$	**1.57**	**0.44**	**2.05**	**0.97**	**84.14**
	c.v.	**2.22**	**3.31**	**4.10**	**1.62**	**9.72**
Winter varieties	Zyta	72.47	14.0	51	61.69	1200
	Korweta	73.57	13.6	49	61.62	1250
	Finezja	75.43	12.3	47	59.58	950
	Tonacja	76.24	11.5	49	61.15	1020
	Mewa	76.14	11.7	45	57.55	720
	Slawa	73.89	12.1	41	57.18	610
	$\bar{x}$	**74.62**[a]	**12.5**[a]	**47**[a]	**59.79**[a]	**958**[a]
	$\hat{s}$	**1.54**	**1.02**	**3.58**	**2.03**	**255**
	c.v.	**2.06**	**8.19**	**7.61**	**3.40**	**26.6**

$\bar{x}$, mean value; $\hat{s}$, standard deviation; c.v., coefficient of variability (%); FWA, water absorption. Mean values for each class of wheat flour with the same letter are not significantly different at $p < 0.05$.

Brabender Quadrumat Junior (Brabender OHG, Duisburg, Germany). The flour yields and their basic characteristics are presented in Table 1. The flours obtained immediately after milling were analysed for free lipids and carotenoids.

2.2. Free lipids extraction and fractionation

The content of free lipids was analysed by the Soxhlet method with petroleum ether according to Polish Standard PN-73/R-66164. Crude lipid extract was dissolved in 25 cm^3 of chloroform and was then fractionated using SPE prepacked columns (Bond Elut Glass-SI, 1000MG 6ML, Varian). Column conditioning and lipid separation were done according to Ohm and Chung (1999) using a vacuum manifold (Baker). The non-polar lipid, glycolipid and phospholipid fractions were separated by elution with 10 cm^3 of a chloroform–acetone mixture (4:1, v/v), followed by 15 cm^3 of an acetone–methanol mixture (9:1, v/v) and 10 cm^3 of methanol, respectively. The solvent flow rate was stabilized at 0.7 ml/min. Each fraction was evaporated under pressure and nitrogen to dryness at 40 °C and weighed. The recovery of fractionated lipids was in the range 96–100% of extracted free lipids. The efficiency of the SPE procedure for separating main classes of wheat lipids was 100% (purity of each fraction was analysed by thin-layer chromatography in preliminary studies).

2.3. Free glycolipids analysis

Glycolipids were re-dissolved in 10 cm^3 of chloroform and separated by thin layer chromatography using pre-coated TLC plates Silgur-25 UV_{254} (Machery-Nagel, Germany) of dimensions 20×20 cm, 0.25 cm layer thickness, with isolated concentration zone. Before analyses, the plates were developed in a mixture of chloroform: methanol (2:1) and activated at 130 °C/1 h. On such prepared plates, 25 μl of sample were placed and developed according to Prieto, Ebri, and Collar (1992) in two solvent systems: I: chloroform:acetone:acetic acid:water (10:90:2:3) and II: ethyl ether:acetic acid (99:1). The developed plates were sprayed with a 25% solution of sulphuric acid supplemented with 0.6% potassium (II) chromate and heated at 130 °C for 1 h. Next, the plates were scanned (Plustek Optic Pro UT 24 scaner at 600 dpi) and measured densitometrically using LUCIA G v. 4.8 software. Glycolipids were quantified by multiplying the mean gray level and area of each spot. The qualitative and quantitative identification of the obtained spots was carried out with the use of the MGDG and DGDG standards (Sigma) and by comparing the results with the separation model presented in the paper by Prieto et al. (1992).

2.4. Fatty acid analysis

The fatty acid composition of free lipids was analysed according to Polish Standard PN-EN-ISO-5508:1996. The conditions of separation were as follows: CHROM 5 GC with FID detector, a glass column filled with GP3% SP-2310/2% SP-2300 on 100/120 Chromosorb W AW, injection, oven and detector temperatures were: 230, 210 and 250 °C, respectively. Fatty acid methyl esters (FAME) were prepared according to Zadernowski and Sosulski (1978) using a mixture of chloroform:methanol:sulphuric acid (100:100:1, v/v/v). Fatty acids were identified and quantified using a reference mix of rapeseed oil fatty acids (Supelco).

2.5. Carotenoids extraction and analysis

The isolation of carotenoids was performed as described by Chen, Peng, and Chen (1995). The pigments were extracted from 30 g of flour using 30 cm^3 mixture of hexane, acetone, 99.8% ethanol and toluene in the proportions of 10:7:6:7 v/v/v/v and 6 cm^3 of 40% KOH in methanol. In order to prevent carotenoid oxidation, 4 cm^3 of 0.1% BHT were used. The solution was saponified at room temperature in the dark for 16 h. Next, the sample was supplemented with 30 cm^3 of hexane and made up to 200 cm^3 with 10% Na_2SO_4. The upper phase was collected and the lower phase was thrice rinsed with 10 cm^3 of hexane. All supernatants were evaporated at 40 °C under a nitrogen stream and dissolved in 2 cm^3 of methanol:dichloromethane (45:55 v/v) and filtered through Rezist 30/0.2. The purified extracts were separated and quantified at 25 °C by HPLC (Hewlett–Packard 1050) equipped with YMC-C30 10×4.6 mm, 3 μm precolumn and YMC-C30 250×4.6 mm, 5 μm column (YMC-Europe GMBH). The pigments were eluted by methanol:MTBE (89:11) at a flow rate of 1 ml/min. The absorbance was measured at the wavelength of 450 nm. Carotenoids were identified, based on retention times of the lutein and α- and β-carotene true standards (Sigma) and by comparing the UV–Visible absorption spectra with previously published results. The hypsochromic shift, occurrence of significant absorption in ultraviolet region, the *Q* ratio (ratio of maximum absorption of *trans* to *cis*-peak) (Tai & Chen, 2000) and the % III/II ratio (height of longest wavelength absorption peak), designated III, to that of middle absorption peak, designated II, taking the minimum between the two peaks as baseline, multiplied by 100 (Rodriguez-Amaya, 2001) were analysed.

Total carotenoid content was measured according to Rodriguez-Amaya (2001). The absorption coefficient ($A^{1\%}_{1\,cm}$) of lutein in ethanol at 445 nm (2 550) was taken.

2.6. Flour quality analysis

The flours were also tested for moisture and protein contents (Polish Standard PN-91/A-74010 and PN-75/A-04018, respectively), Zeleny test (ISO 5529), amylograph viscosity (according to ISO 7973 in Amylograph Model. No. 800145, Brabender OHG, Duisburg, Germany) and water absorption (AACC method 54-21 in a Brabender farinograph OHG, Duisburg, Germany). These measurements were performed to check the wheat sample quality and confirm that the kernels were not damaged by hydrolytic enzymes.

2.7. Statistical analysis

Quality control of lipid fractionation on silica SPE columns and carotenoid extraction and detection was intra-laboratory tested in preliminary studies using known standards. Significant differences between spring and winter wheat flours were calculated using Statistica 6.0 PL software (StatSoft Tulsa, USA) at a significance level of $p < 0.05$. Analysis of variance with Duncan tests was conducted for each variety sampled, in triplicate, for lipid and carotenoids measurements.

3. Results and discussion

3.1. Free lipids

Free lipid composition is mainly variety- and environment-dependent (Chung & Ohm, 1997; Simmonds, 1989) but it can be modified by the milling technology (Barnes, 1983) and by kernel maturity at harvest (Grzesiuk & Kulka, 1988; Skarsaune, Youngs, & Gilles, 1970). The kind of solvent used for extraction also plays an important role (Chung & Ohm, 1997; MacRitchie & Gras, 1973). Such a wide variety of modification factors makes it difficult to compare the results obtained by different authors.

In the present paper, this variability was limited to the effect of wheat class (spring/winter) while the cultivation environment, milling technology, lipid extraction and separation techniques were identical. Free lipid contents in flour and their separation into fractions of varied polarity are presented in Table 2. Spring wheat flours were about 17% richer in total lipids, especially in their non-polar fraction. Considering the fact that at the same time they had a more than 4% lower milling yield (Table 1), this indicates a greater concentration of non-polar lipids in the endosperm of these wheats. The mean percentage of non-polar fraction was similar in both groups of wheat with a similar variability for these varieties (79.9–85.6% and 78.1–82.9%, for spring and winter wheats, respectively). According to the literature, the percentage of non-polar fraction can range from ca. 70 to 90% (Bekes et al., 1986; Karpati et al., 1990; Ohm & Chung, 2002). Bekes et al. (1986) also showed that the highly variable free lipid contents (depending on cultivation site the total free lipids content differed 2-fold in the same wheat variety) is accompanied by a relatively low variability in the proportions of their components.

The sources of non-polar lipids in flour are small discrete oil storage bodies, called spherosomes. Spherosomes are deposited mainly in germ and aleurone and to a much smaller extent in mature starchy endosperm

Table 2
Content and composition of flour free lipids

	Variety	TFL mg/100 g	NL		GL		PhL	
			mg/100 g	% of TFL	mg/100 g	% of TFL	mg/100 g	% of TFL
Spring varieties	Zebra	1343	1073	79.9	215	16.0	55	4.1
	Jasna	1276	1060	83.1	166	13.0	50	3.9
	Olimpia	1251	1046	83.6	147	11.8	58	4.6
	Koksa	1316	1055	80.2	206	15.7	55	4.2
	Opatka	1242	1063	85.6	134	10.8	45	3.6
	$\bar{x}$	**1285.6**[a]	**1059**[a]	**82.4**[a]	**174**[a]	**13.5**[a]	**52.6**[a]	**4.1**[a]
	$\hat{s}$	**43.0**	**9.96**	**2.41**	**35.7**	**2.32**	**5.13**	**0.37**
	c.v.	**3.35**	**0.94**	**2.92**	**20.6**	**17.2**	**9.75**	**9.03**
Winter varieties	Zyta	1026	801	78.1	196	19.1	29	2.8
	Korweta	1173	954	81.3	186	15.9	33	2.8
	Finezja	1149	934	81.3	183	15.9	32	2.8
	Tonacja	1043	850	81.5	160	15.3	33	3.2
	Mewa	1090	904	82.9	158	14.5	28	2.6
	Slawa	1132	926	81.8	167	14.8	39	3.4
	$\bar{x}$	**1102**[b]	**894.8**[b]	**81.2**[a]	**175**[a]	**15.9**[a]	**32.3**[b]	**2.9**[b]
	$\hat{s}$	**59.3**	**58.2**	**1.61**	**15.5**	**1.66**	**3.88**	**0.30**
	c.v.	**5.38**	**6.50**	**1.98**	**8.87**	**10.4**	**12.0**	**10.4**

$\bar{x}$, mean value; $\hat{s}$, standard deviation; c.v., coefficient of variability (%). TFL, total free lipids; NL, non-polar lipids; GL, glycolipids; PhL, phospholipids. Mean values for each class of wheat flour with the same letter are not significantly different at $p < 0.05$.

(Hargin, Morrison, & Fulcher, 1980). Their chemical compositions vary to a small extent between different plant species (Tzen & Huang, 1992). In the case of maize kernel, lipid bodies are composed of 97% triglycerides, constituting their hydrophobic core and about 1% phospholipids. building a ''half-unit'' membrane of these organelles (Tzen & Huang, 1992). The lipids of this monolayer consist of three main fractions (PC, PI and PE); however, the percentage of the first two fractions is considerably greater (about 37% each) (Moreau et al., 1998).

The content of the total glycolipids was more connected with wheat variety than with its class (Table 2). The slightly higher mean percentages of free lipids of flour from winter wheat was accompanied by smaller variability within varieties. Spring varieties also varied

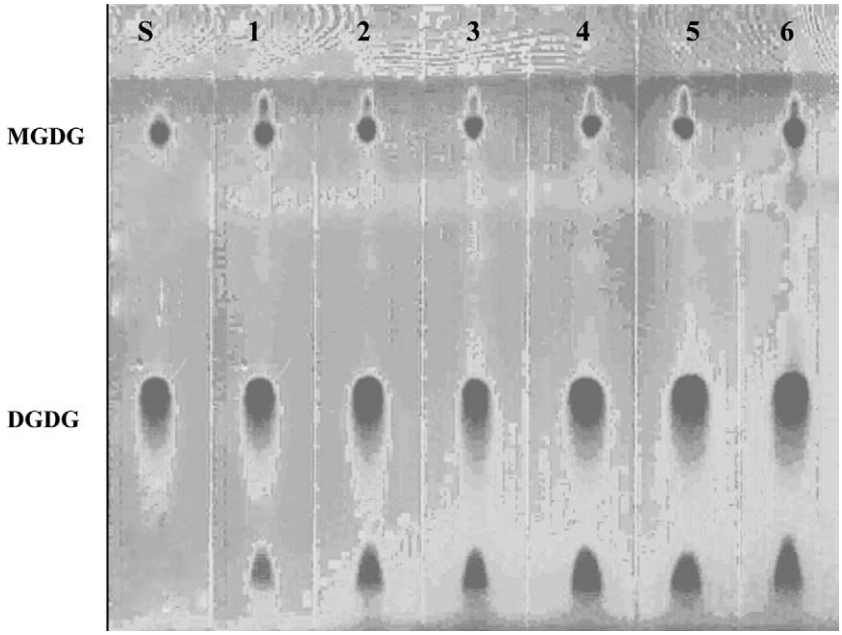

Fig. 1. Thin-layer chromatogram of free glycolipids from winter wheat flours (separation in two-solvent systems: chloroform, acetone, acetic acid, and water (10:90:2:3) to 13 cm and ethyl ether and acetic acid (99:1) to 16 cm). S-line of standards (DGDG-digalactosyldiglycerol and MGDG-monogalactosyldiglycerol), 1–6-lines of analysed flours.

Table 3
Content and composition of flour glycolipids

	Variety	DGMG		DGDG		MGMG		MGDG	
		% of GL	mg/100 g	% of GL	mg/100 g	% of GL	mg/100 g	% of GL	mg/100 g
Spring varieties	Zebra	8.46	18	51.8	111	18.4	40	21.4	46
	Jasna	7.70	13	60.0	100	12.9	21	19.4	32
	Olimpia	8.83	13	62.4	92	8.10	12	20	30
	Koksa	8.02	17	61.5	127	9.66	20	20.8	43
	Opatka	9.60	13	53.3	71	19.8	26	17.8	24
	$\bar{x}$	**8.52**[a]	**15**[a]	**57.8**[a]	**100**[a]	**13.8**[a]	**24**[a]	**20.0**	**35**[a]
	$\hat{s}$	**0.74**	**2.49**	**4.91**	**21.0**	**5.18**	**10.4**	**1.44**	**9.22**
	c.v.	**8.68**	**16.60**	**8.49**	**21.0**	**37.63**	**43.1**	**7.18**	**26.3**
Winter varieties	Zyta	12.33	24	63.5	125	10.33	20	13.2	26
	Korweta	13.23	25	57.3	107	15.35	29	14.1	26
	Finezja	13.79	25	65.9	121	8.57	16	11.7	21
	Tonacja	13.83	22	63.5	102	7.62	12	15.1	24
	Mewa	14.37	23	63.9	101	7.86	12	13.9	22
	Slawa	10.20	17	61.2	102	10.5	18	18.12	30
	$\bar{x}$	**12.96**[b]	**23**[b]	**62.5**	**109**[a]	**10.0**[a]	**18**[a]	**14.4**[b]	**25**[a]
	$\hat{s}$	**1.52**	**3.01**	**2.98**	**10.6**	**2.87**	**6.34**	**2.16**	**3.25**
	c.v.	**11.71**	**13.09**	**4.76**	**9.74**	**28.6**	**35.2**	**15.0**	**13.0**

$\bar{x}$, mean value; $\hat{s}$, standard deviation; c.v., coefficient of variability (%). DGMG, digalactosylmonoglycerol; DGDG, digalactosyldiglycerol; MGMG, monogalactosylmonoglycerol; MGDG, monogalactosyldiglycerol. Mean values for each class of wheat flour with the same letter are not significantly different at $p < 0.05$.

to a greater extent in content of glycolipids (134–215 mg/100 g of flour) than did winter wheats (158–196 mg/100 g of flour).

Literature on which wheat class is richer in free glycolipids is ambiguous. Their greater content in the winter wheat flours can be speculated by comparing the results of Ohm and Chung (2002) and Bekes et al. (1986). The first authors find that free glycolipids constitute from 12.8% to 16.6% of HWW wheat flour and, the second, 7.7–10.2% of HWS wheat flour. According to Matsoukas and Morrison (1991), winter bread wheats grown in Greece contain about 15.5% glycolipids in the free lipid fraction. It seems, however, that different analytical procedures for lipid extraction and separation, applied in the cited studies, cannot be the basis for concluding that free lipids of winter wheat flour contains more glycolipids. As long as 30 years ago, MacRitchie and Gras (1973) suggested that the terms "free" lipids may be misleading because, in flour, there appears to be a portion of total lipid associated with different compounds, which may be extracted to a greater or lesser degree by different cold solvents. More distinct comparison of these wheat classes was presented by Bell et al. (1987) who reported that spring wheats were richer in free glycolipids. This shows that the question of glycolipid connection with wheat specific class is not fully explained. Further research should be performed on wheat samples of similar quality, especially in protein content and composition. More synonymous results would probably be obtained by extraction of all glycolipids. Glycolipids are deposited in the membrane of amyloplasts, so number and magnitude of starch granules can strongly influence their content.

Phospholipids constituted the smallest percentage of flour free lipids and ranged from 2.6% to 4.6% (Table 2). A greater content of FPhL in spring wheat can be associated with a greater content of non-polar lipids (likely greater percentage of spherosomes). However, looking at the spherosome membrane, only about 1% of the FNL mass, which was about 10 mg phospholipids, originated from this. Thus, other cellular biomembranes were the source of the remaining FPhL. They can also originate from mechanically damaged starch, in which they occur as integral components (Morrison, 1995).

Polar lipids in kernels are not a storage material, but build varied cellular membranes. The degree of their extraction with cold non-polar solvents can be related to membrane destruction level. Such destruction occurs naturally in a kernel at endosperm programmed cell death (PCD) during kernel maturation and as result of different kinds of stresses (Young & Gallie, 2000). As a result of these processes, lamellar biomembranes of starchy endosperm, composed partly of polar lipids, become non-lamellar, mainly hexagonal and/or cubic (Marion & Clark, 1995; Welti et al., 2002). In addition, the stress of mechanical endosperm destruction during kernel grinding (measured as, e.g., the amount of damaged starch or mean flour particle diameter) may modify lipid susceptibility to extraction.

3.2. Free glycolipids composition

Among the free glycolipids, four main fractions were detected and identified as DGMG, DGDG, MGMG and MGDG (Table 2, Fig. 1) by comparing

Table 4
Fatty acids compositions of flour free lipids

	Variety	Fatty acid (%)						
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Others
Spring varieties	Zebra	19.1	0.02	0.68	13.5	63.0	3.38	0.34
	Jasna	18.9	0.02	0.57	14.1	62.2	3.72	0.48
	Olimpia	19.8	0.04	0.64	13.7	62.8	2.81	0.27
	Koksa	20.0	0.02	0.68	14.2	62.0	2.72	0.46
	Opatka	19.6	0.01	0.67	15.1	62.8	1.80	0.04
	$\bar{x}$	**19.5**	**0.02**[a]	**0.65**[a]	**14.1**[a]	**62.6**[a]	**2.89**[a]	**0.32**[a]
	$\hat{s}$	**0.49**	**0.01**	**0.05**	**0.60**	**0.43**	**0.73**	**0.18**
	c.v.	**2.52**	**54.8**	**7.17**	**4.22**	**0.69**	**25.4**	**55.6**
Winter varieties	Zyta	19.4	0.04	0.69	11.5	63.4	4.74	0.21
	Korweta	19.6	0.05	0.54	11.2	63.3	4.97	0.38
	Finezja	19.8	0.01	0.48	11.2	63.4	4.59	0.54
	Tonacja	19.3	0.02	0.50	11.7	64.2	4.05	0.24
	Mewa	19.4	0.01	0.49	11.8	63.5	4.28	0.50
	Slawa	20.7	0.01	0.47	11.3	63.2	3.80	0.63
	$\bar{x}$	**19.7**[a]	**0.02**[a]	**0.53**[b]	**11.5**[b]	**63.5**[b]	**4.41**[b]	**0.42**[a]
	$\hat{s}$	**0.51**	**0.02**	**0.08**	**0.26**	**0.37**	**0.44**	**0.17**
	c.v.	**2.58**	**87.6**	**15.6**	**2.29**	**0.58**	**10.0**	**40.3**

$\bar{x}$, mean value; $\hat{s}$, standard deviation; c.v., coefficient of variability (%). Mean values for each class of wheat flour with the same letter are not significantly different at $p < 0.05$.

with standards (DGDG and MGDG) and literature (Prieto et al., 1992). The DGDG predominated and constituted, on average, approx. 58% and 63% of FGL from spring and winter wheat, respectively. Spring wheats were richer in MGDG, both in mass and percentage. The percentage sums of both these diacylglycerols were similar in both wheat classes and approx. 77%. According to Ohm and Chung (2002), DGDG constitute on average 42.5% and MGDG 17.5% of the composition of FGL from HWW wheat cultivated in the US. Studies by Karpati et al. (1990) into wheat cultivated in Hungary found a greater similarity of its glycolipid composition to that presented in the present paper. These authors showed that, in some cases, the total content of the two main fractions (DGDG and MGDG) reached 90%. The source of both glycolipid fractions is exclusively associated with plastid membranes (Moreau et al., 1998). The presence of galactosylomonoglycerol fractions in flour indicates the activity of lipolytic acyl hydrolases in mature or maturing wheat endosperm (Barnes, 1983).

3.3. Free lipid fatty acid composition

An analysis of fatty acid composition of free lipids did not reveal any significant diversification in either

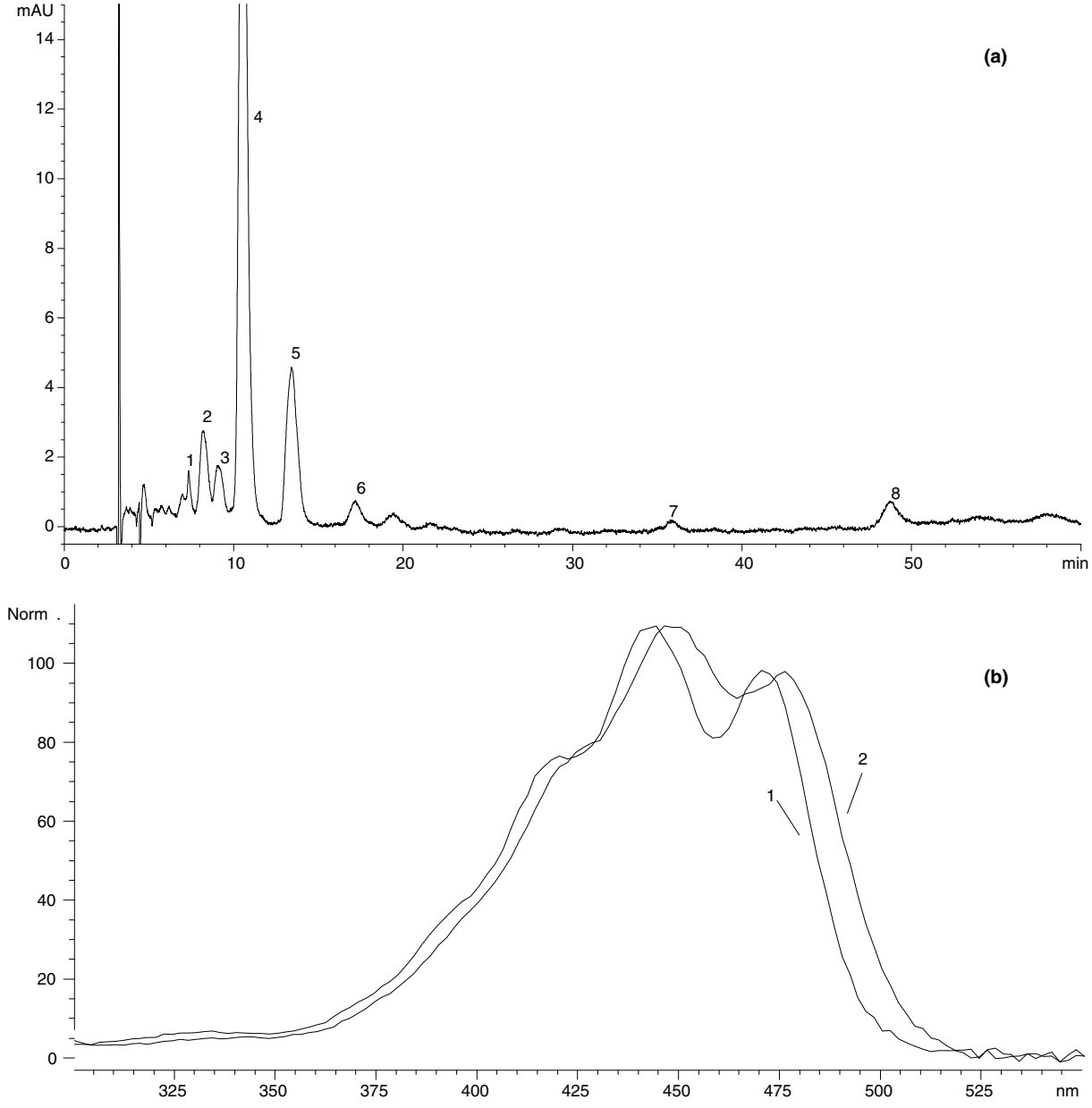

Fig. 2. An example of HPLC chromatogram of carotenoids in wheat flour of variety Olimpia (elution with methanol:MTBE (89:11) at a flow rate of 1 ml/min at 25 °C on YMC-C30 250 × 4.6 mm, 5 μm column, detection at 450 nm). (a) Peaks identification: 1/2. 13/13′-*cis*-lutein, 3. 13-*cis*-zeaxanthin, 4. lutein, 5. zeaxanthin, 6.9-*cis*-lutein, 7. α-carotene, 8. β-carotene; (b) Absorbance spectra of main components: 1. lutein, 2. zeaxanthin.

Table 5
Composition of carotenoids in spring and winter wheat flour

	Variety	Content (μg/100 g of d.b.)	Composition (%)										
			13/13′-*cis*-lutein	13-*cis*-zeaxanthin	Lutein	Zeaxanthin	9-*cis*-lutein	α-carotene	β-carotene	Unidentified	Total lutein	Total zeaxanthin	*L*/*Z* ratio
Spring varieties	Zebra	334	11.3%	6.26	59.9	13.00	3.31	2.30	2.73	1.27	74.4	19.3	3.87
	Jasna	248	11.1	5.81	64.2	14.0	3.63	0.00	0.00	1.31	78.9	19.8	3.98
	Olimpia	397	8.23	4.40	59.4	16.1	3.72	2.81	4.45	0.89	71.3	20.51	3.48
	Koksa	301	12.0	6.55	60.5	15.0	4.78	0.00	0.00	1.10	77.4	21.6	3.59
	Opatka	481	10.1	5.61	65.6	12.4	2.65	0.00	2.70	0.93	78.3	18.0	4.34
	$\bar{x}$	**352**[a]	**10.54**[a]	**5.73**[a]	**61.9**[a]	**14.1**[a]	**3.62**[a]	**1.02**	**1.98**[a]	**1.10**[a]	**76.1**	**19.8**[a]	**3.85**[a]
	$\hat{s}$	**90**	**1.46**	**0.83**	**2.80**	**1.49**	**0.77**	**1.41**	**1.94**	**0.19**	**3.15**	**1.32**	**0.34**
	c.v.	**25.57**	**13.9**	**14.5**	**4.51**	**10.6**	**21.4**	**138**	**98.1**	**17.4**	**4.14**	**6.65**	**8.87**
Winter varieties	Zyta	191	10.30	5.72	67.7	12.4	2.80	0.00	0.00	1.00	80.9	18	4.48
	Korweta	266	7.54	3.69	70.7	11.9	2.13	0.00	3.18	0.86	80.3	15.6	5.14
	Finezja	265	8.59	4.36	72.9	11.4	1.87	0.00	0.00	0.88	83.3	15.8	5.27
	Tonacja	211	7.58	3.47	72.2	13.6	2.12	0.00	0.00	1.04	81.9	17.04	4.81
	Mewa	164	8.79	2.72	67.2	21.3	0.00	0.00	0.00	0.00	76.0	24.01	3.16
	Slawa	359	7.95	3.83	72.0	11.21	2.50	0.00	1.65	0.88	82.4	15.0	5.48
	$\bar{x}$	**242**[b]	**8.46**[b]	**3.97**[b]	**70.5**[b]	**13.6**[a]	**1.90**[b]	**0.00**	**0.81**[a]	**0.78**[a]	**80.8**[b]	**17.60**[a]	**4.72**[a]
	$\hat{s}$	**70**	**1.04**	**1.01**	**2.40**	**3.84**	**0.99**	**0.00**	**1.34**	**0.39**	**2.59**	**3.33**	**0.84**
	c.v.	**28.9**	**12.3**	**25.5**	**3.41**	**28.2**	**51.9**	–	**166**	**49.9**	**3.21**	**18.9**	**17.84**

$\bar{x}$, mean value; $\hat{s}$, standard deviation; c.v., coefficient of variability (%). Mean values for each class of wheat flour with the same letter are not significantly different at $p < 0.05$.

class of wheat (Table 3). The fatty acids of spring wheat contained more oleic and slightly less linoleic and linolenic acids. Armanino, De Acutis, and Festa (2002) found lower levels of linoleic acid (ca. 57.4%) in 54 samples of *Triticum aestivum* grown in Germany, Italy and the UK. Davis et al. (1980) also obtained similar results for wheats cultivated in the US. Apart from genetic factors, the fatty acid composition is also determined by climatic conditions. Welch (1975) reported that a colder growing temperature can result in increased lipid content and an increased amount of unsaturation in wheat cultivated in the US. This is either not in agreement with our results (year 2003 in Poland was extremely warm and dry) or varieties cultivated in Poland are characterized by higher contents of unsaturated fatty acids.

3.4. Flour carotenoids

The carotenoid content and composition of analysed flours are presented in Table 4 and Fig. 2. It was shown that the mean content of their sums is greater in spring wheat flour (on average 352 against 242 μg/100 g of flour, dry basis); however, there were varieties that overlapped both classes. The results are similar to those presented by other authors (Abdel-Aal et al., 2002; Heinonen et al., 1989; Kaneko et al., 1995; Zandomeneghi, Festa, & Carbonaro, 2000). A much smaller content of these pigments in wheat was reported by Humphries and Khachik (2003) but they analysed only two samples of mature wheat kernels. Previous studies (Konopka, Kozirok, & Rotkiewicz, 2004) showed that the content of carotenoids in Polish winter wheat flour was from 156 to 228 μg/100 g. The present results showed a higher content of carotenoids in the flour of this class of wheat. This can be explained, along with changes in fatty acids composition, by specific plant growth and kernel maturing conditions. Both growing plants and moulded kernels were subjected to heat and light stresses that could induce defence mechanisms in which carotenoids are known to be efficacious compounds against photooxidation (Bartley & Scolnik, 1995). Recently, Rabbani, Beyer, v.Lintig, Hugueney, and Kleinig (1998) reported that the carotenogenic pathway is not maximally active under normal conditions (research on alga Dunaliella Bardawil). Overproduction β-carotene occurs under stress conditions (e.g., high light intensity) and is accompanied by higher accumulation of oil droplets. So, this could explain the larger contents both free lipids and carotenoids in flour of spring wheat, but further research on this subject is necessary.

The main compound of this group of pigments was lutein, whose total percentage in the form of different isomers ranged from 71.3% to 83.3% and was slightly lower for spring wheat flour. Previous investigations (Konopka et al., 2004) found that the lutein in Polish winter wheat flour accounted for ca. 95% of all carotenoids. The differences can be due to modification of HPLC analyses conditions. In the present study, a C_{30} instead of C_{18} phase column was used, which is more selective for efficient separation of carotenoids (Dachtler, Kohler, & Albert, 1998; Rodriguez-Amaya, 2001). Lutein, in the form of a *trans*-isomer, constituted about 2/3 of all carotenoids. The remaining important compounds were zeaxanthin and 13-*cis* and 13′-*cis*-isomers of both main fractions (lutein and zeaxanthin). The presence of α- and β-carotene was noted in only a few samples and their content reached maximally 4.45% of the total carotenoids. The presence of β-carotene in flour can indicate its contamination with the bran layer. This is in agreement with results of Heinonen et al. (1989) who reported that the content of β-carotene decreased from 5.3 to below 1 μg/100 g of flour with ash content in flour decreasing from 1.3% to 0.7%. (See Table 5).

The carotenoid biosynthetic pathway, presented in the work of Park, Kreunen, Cuttriss, DellaPenna, and Pogson (2002), shows that lutein and zeaxanthin have different precursors, α- and β-carotene, respectively. The pathway of carotenoid synthesis is quite well understood (Park et al., 2002) but their regulation is controlled by a yet-unidentified developmental signal and also their function in non-photosynthetic tissue, such as wheat endosperm, is not clear (Bartley & Scolnik, 1995). Zeaxanthin is known to be a precursor of abscisis acid (ABA), a phytohormone that modulates developmental and stress processes (Bartley & Scolnik, 1995) and it is responsible for grain maturation.

4. Conclusions

Wheat native lipids are minor components of flour but they play significant roles in baking. Wheat flour, consumed in the form of bread, cakes, pasta, and other snacks, is also an important source of unsaturated fatty acids and carotenoids. The results suggest that free lipid and carotenoid contents have to be partly regulated by wheat vernalization genes. Flours milled from spring wheat kernels of plants cultivated in the same place and subjected to the same weather conditions differed in free lipids (especially in non-polar fraction and phospholipids), fatty acids and carotenoid composition. However, both groups of compounds can be significantly changed by stresses during plant growth and kernel maturation (so-called environmental variability). Prediction of wheat kernel composition can help improve its utilization and also human health.

References

Abdel-Aal, E. S., Young, J. C., Wood, P. J., Rabalski, I., Hud, P., Falk, D., et al. (2002). Einkorn: a potential candidate for developing high lutein wheat. *Cereal Chemistry, 79*, 455–457.

Armanino, C., De Acutis, R., & Festa, M. R. (2002). Wheat lipids to discriminate species, varieties, geographical origins and crop years. *Analytica Chimica Acta, 454*, 315–326.

Barnes, P. J. (1983). *Lipids in cereal technology*. London: Academic Press.

Bartley, G. E., & Scolnik, P. A. (1995). Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *The Plant Cell, 7*, 1027–1038.

Bekes, F., Zawistowska, U., Zillman, R. R., & Bushuk, W. (1986). Relationship between lipid content and composition and loaf volume of 26 common spring wheats. *Cereal Chemistry, 63*, 327–331.

Bell, B. M., Daniels, D. G. H., & Fearn, T. (1987). Lipid compositions, baking qualities and other characteristics of wheat varieties grown in the UK. *Journal of Cereal Science, 5*, 277–286.

Bone, R. A., Landrum, J. T., Dixon, Z., Chen, Y., & Llerena, C. M. (2000). Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Experimental Eye Research, 71*, 239–245.

Chen, B. H., Peng, H. Y., & Chen, H. E. (1995). Changes of carotenoids, color and vitamin A contents during processing of carrot juice. *Journal of Agricultural and Food Chemistry, 43*, 1912–1918.

Chung, O. K., & Ohm, J. B. (1997). Wheat lipids as a quality determinant. In *Proceedings of the international wheat quality conference, Manhattan, Kansas, USA, 18–22 May 1997* (pp. 83–100).

Chung, O. K., Pomeranz, Y., & Finney, K. F. (1982). Relation of polar lipid content to mixing requirement and loaf volume potential of hard red winter wheat flour. *Cereal Chemistry, 59*, 14–20.

Dachtler, M., Kohler, K., & Albert, K. (1998). Reversed-phase high-performance liquid chromatographic identification of lutein and zeaxanthin stereoisomers in bovine retina using a C_{30} bonded phase. *Journal of Chromatography B, 720*, 211–216.

Davis, K. R., Litteneker, N., Le Tourneau, D., Cain, R. F., Peters, L. J., & McGinnis, J. (1980). Evaluation of the nutrient composition of wheat. I. Lipid constituents. *Cereal Chemistry, 57*, 178–184.

Grzesiuk, S., & Kulka, K. (1988). *Biologia ziarniaków zbóż*. Warszawa, Poland: PWN.

Hargin, K. D., Morrison, W. R., & Fulcher, R. G. (1980). Triacylglicerol deposits in the starchy endosperm of wheat. *Cereal Chemistry, 57*, 320–325.

Heinonen, M., Olliainen, V., Linkola, E., Varo, P., & Koivistoinen, P. (1989). Carotenoids and retinoids in finnish foods: cereal and bakery products. *Cereal Chemistry, 66*, 270–273.

Humphries, J. M., & Khachik, F. (2003). Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of Agricultural and Food Chemistry, 51*, 1322–1327.

Kaneko, S., Nagamine, T., & Yamada, T. (1995). Esterification of endosperm lutein with fatty acids during the storage of wheat seeds. *Bioscience Biotechnology Biochemistry, 59*, 1–4.

Karpati, E. M., Bekes, F., Lasztity, R., & Oersi, F. (1990). Investigation of the relationship between wheat lipids and baking propertes. *Acta Alimentaria, 19*, 237–260.

Konopka, I., Kozirok, W., & Rotkiewicz, D. (2004). Lipids and carotenoids of wheat grain and flour and attempt of correlating them with digital image analysis of kernel surface and cross-sections. *Food Research International, 37*, 429–438.

Lepage, M., & Sims, R. P. A. (1968). Carotenoids of wheat flour: their identification and composition. *Cereal Chemistry, 45*, 600–604.

MacRitchie, F., & Gras, P. W. (1973). The role of lipids in baking. *Cereal Chemistry, 50*, 292–302.

Marion, D., & Clark, D. C., (1995). Wheat lipids and lipid-binding proteins: structure and function. In J. D. Schofield (Ed.), *The proceedings of a conference organised by the Royal Society of Chemistry, UK* (pp. 245–260).

Matsoukas, N., & Morrison, W. R. (1991). Breadmaking quality of ten Greek breadwheats. II. Relationships of protein, lipid and starch components to baking quality. *Journal of the Science of Food and Agriculture, 55*, 87–101.

Moreau, P., Bessoule, J. J., Mongrand, S., Testet, E., Vincent, P., & Cassagne, C. (1998). Lipid trafficking in plant cells. *Progress in Lipid Research, 37*, 371–391.

Morrison, W. R., Law, C. N., Wylie, L. J., Coventry, A. M., & Seekings, J. (1989). The effect of group 5 chromosomes on the free polar lipids and breadmaking quality of wheat. *Journal of Cereal Science, 9*, 41–51.

Morrison, W. R., (1995). Starch lipids, starch granule structure and properties. In J. D. Schofield (Ed.), *The proceedings of a conference organised by the Royal Society of Chemistry, UK* (pp. 261–270).

Nelson, J. C., Sorrels, M. E., Van-Deynze, A. E., Lu, Y. H., Atkinson, M., Bernard, M., et al. (1995). Molecular mapping of wheat: a major genes and rearrangements in homoeologous groups 4, 5 and 7. *Genetics, 141*, 721–731.

Ohm, J. B., & Chung, O. K. (1999). Estimation of free glycolipids in wheat flour by HPLC. *Cereal Chemistry, 76*, 873–876.

Ohm, J. B., & Chung, O. K. (2000). NIR transmittance estimation of free lipid content and its glycolipid and digalactosylgliceride contents using wheat flour lipid extracts. *Cereal Chemistry, 77*, 556–559.

Ohm, J. B., & Chung, O. K. (2002). Relationships of free lipids with quality factors in hard winter wheat flours. *Cereal Chemistry, 79*, 274–278.

Panozzo, J. F., Hannah, M. C., O'Brien, L., & Bekes, F. (1993). The relationship of free lipids and flour protein to breadmaking quality. *Journal of Cereal Science, 17*, 47–62.

Park, H., Kreunen, S. S., Cuttriss, A. J., DellaPenna, D., & Pogson, B. J. (2002). Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. *The Plant Cell, 14*, 321–332.

Prieto, J. A., Ebri, A., & Collar, C. (1992). Optimized separation of nonpolar and polar lipid classes from wheat flour by solid-phase extraction. *Journal of American Oil Chemists Society, 69*, 387–391.

Rabbani, S., Beyer, P., v.Lintig, J., Hugueney, P., & Kleinig, H. (1998). Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga Dunaliella Bardawil. *Plant Physiology, 116*, 1239–1248.

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*. Washinghton, USA: ILSI (International Life Sciences Institute) Press.

Ruibal-Mendieta, N. L., Delacroix, D. L., & Meurens, M. (2002). A comparative analysis of free, bound and total lipid content on spelt and winter wheat wholemeal. *Journal of Cereal Science, 35*, 337–342.

Simmonds, D. H. (1989). *Wheat and wheat quality in Australia. Chapter11. Fundamental aspects of wheat quality: the lipid fraction*. Australia: CSIRO.

Skarsaune, S. K., Youngs, V. L., & Gilles, K. A. (1970). Changes in wheat lipids during seed maturation. I. Physical and chemical changes in the kernel. *Cereal Chemistry, 47*, 522–531.

Snodderly, D. M. (1995). Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *American Journal of Clinical Nutrition, 62*(Suppl.), 1448S–1461S.

Tai, C.-Y., & Chen, B. H. (2000). Analysis and stability of carotenoids in the flowers of daylily (Hemerocallis) disticha as affected by various treatments. *Journal of Agriculture and Food Chemistry, 48*, 5962–5968.

Tzen, J. T. C., & Huang, A. H. C. (1992). Surface structure and properties of plant seed oil bodies. *The Journal of Cell Biology, 117*, 327–335.

Welch, R. W. (1975). Fatty acid composition of grain from winter and spring sown oats, barley and wheat. *Journal of the Science of Food and Agriculture, 26*, 429–437.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.-E., et al. (2002). Profiling membrane lipids in plant stress responses. *The Journal of Biological Chemistry, 277*, 31994–32002.

Young, T. E., & Gallie, D. R. (2000). Programmed cell death during endosperm development. *Plant Molecular Biology, 44*, 283–301.

Zadernowski, R., & Sosulski, F. (1978). Composition of total lipids in rapeseed. *Journal of the American Oil Chemists' Society, 55*, 870–872.

Zandomeneghi, M., Festa, C., & Carbonaro, L. (2000). Front-surface absorbance spectra of wheat flour: determination of carotenoids. *Journal of Agricultural and Food Chemistry, 48*, 2216–2221.