

Scientific paper

Spectrophotometric Estimation of Total Carotenoids in Cereal Grain Products

Svjetlana Luterotti^{1,*} and Kristina Kljak²¹ Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia² Faculty of Agronomy, University of Zagreb, Zagreb, Croatia

* Corresponding author: E-mail: sluter@pharma.hr

Phone: +385 1 63 94 433, fax: +385 1 48 56 201

Received: 27-01-2010

Abstract

Abstract

Total carotenoids (TC) were determined as a measure of total xanthophylls in grain flours and grits, by new validated spectrophotometric method based on $A_{1\text{cm}}^{1\%}$ -approach. The general analytical procedure is easily adjustable to different samples, the number of extraction steps depending on TC concentration in the sample. Basically, two methods have been suggested: the rapid one for low-TC samples (white corn, wheat, soybean, sorghum) including one to two extractions, and the one for high-TC samples (yellow corn) needing three to four extractions. Method's accuracy was proven against the reference standard material ($102.1 \pm 3.9\%$) and the reference method. Good precision (repeatability and intermediate precision of upto 9% for yellow corn products) and sensitivity with *LOD* (limit of detection) and *LLOQ* (lower limit of quantitation) of 0.2 and 0.6 mg kg⁻¹ TC, resp., were estimated. The method was applied to the control of nutritional value of cereal grain products. TC concentration (in mg kg⁻¹) in the samples ranged from 11–23 in yellow corn flours, 0.7–0.9 in white corn flours, 17–22 in yellow corn grits, 1.1–1.3 in wheat flours, 1.6 in wheat grits and 1.5 in sorghum flour, to 0.9–9.9 in soybean flours.

Keywords: Total carotenoids, corn flour/grits, wheat flour/grits, soybean flour, sorghum flour, spectrophotometry

1. Introduction

Carotenoids constitute a family of pigmented lipophilic compounds that are widely distributed in biological systems. They are synthesized by plants and microorganisms but not animals. They are yellow to red pigments and include non-polar hydrocarbons, carotenes (α -carotene, β -carotene, lycopene) and their oxygenated derivatives, xanthophylls (e.g., monohydroxy, cryptoxanthin and zeinoxanthin, dihydroxy, lutein and zeaxanthin, and polyoxy, violaxanthin and neoxanthin).¹ They are highly physiologically important and protect plants and microorganisms against excessive irradiation. Also, some carotenoids possess provitamin A activity. Carotenoids interact with reactive oxygen species and thus act as free radical quenchers, singlet oxygen scavengers and lipid antioxidants.² Research of their antioxidant activity has implied that higher intake of carotenoids leads to a reduced risk of chronic diseases such as cardiovascular diseases and can-

cer.^{3,4} Fruits and vegetables make a major source of carotenoids in human diet.^{5,6}

High performance liquid chromatography (HPLC) is proven the most powerful analytical tool in the field of carotenoid research especially in complex samples, followed by solvent extraction/spectrophotometry, chromatography with supercritical fluid, etc.^{e.g.,7,8,9,10} Despite the fact that there is a huge amount of reports on carotenoids analyses in vegetables, fruits and clinical samples there are just few devoted to analyses of cereals crops and food grains.

Carotenoids are relatively abundant in sweet corn; therefore their quantification is very important from nutritional point of view. Analyses of carotenoids in corn and sorghum grains were reported by Blessin,^{11a} in corn by Weber,¹² Kurilich & Juvik,¹³ Hulshof et al.¹⁴ and Muzhingi et al.,¹⁵ in corn, durum wheat and other cereals by Panfili et al.,¹⁶ in sweet potato, cassava and corn by Rodriguez-Amaya & Kimura¹⁷ and Kimura et al.,¹⁸ in durum

wheat by Hentschel et al.¹⁹ and in red sorghum and other grains by Choi et al.²⁰

In this article we are proposing new, reliable spectrophotometric method for determination of total carotenoids (TC) in different cereal grain flours and grits, and characterizing it by the figures of merit.

2. Experimental

2. 1. Chemicals and Samples

Samples analyzed were sixteen commercial samples of yellow corn, wheat and soybean flours and grits purchased in the grocery stores, and four samples obtained from the Faculty of Agronomy (University of Zagreb, Croatia) (white corn flours and sorghum flour). All samples were stored at room temperature and protected from light.

In total 20 samples were analyzed. These were: three white corn flours (WCF1-WCF3), seven yellow corn flours (YCF1-YCF7), four yellow corn grits (YCG1-YCG4), two wheat flours (WF1-WF2), one wheat grits (WG1), two soybean flours (SF1-SF2), one sorghum flour (SGF1). Pre-cooked white corn flour (WCF1) served as a real blank.

The coarse samples were milled prior to analysis (YCF2, YCF3, YCF6, YCG1, YCG2, YCG3, YCG4).

Hexane (Lach-Ner, Neratovice, Czech Republic), acetone (Kemika, Zagreb, Croatia) and ethanol 96% (Kemika) were of pro analysi purity. Hexane used in all experiments contained 0.025% 2,6-di-*tert*-butyl-4-methylphenol (BHT, purum \geq 99%, Fluka, Buchs, Switzerland).

Certified reference material BCR®-485 (EC-JRC-IRMM, Geel, Belgium) was stored at -30 °C. Before analysis it was equilibrated to the room temperature and rehydrated with water in a ratio 1:20.

2. 2. Instruments

Agilent 8453 spectrophotometer (Hewlett-Packard, Waldbronn, Germany) equipped with PC-HP 845x UV-Visible system was used. It was recalibrated prior to use.

2. 3. Analytical Procedures

TC concentration assay was performed as follows. The homogenized sample, accurately weighed (2 g) was triturated in a porcelain crucible with 10 mL 75% acetone, then transferred into an Erlenmeyer flask with another portion of 10 mL 75% acetone, left overnight at room temperature and agitated for 5 min. (Alternatively, short hydration procedure at room temperature with 30 min stirring may be carried out.)

Hexane (5 mL) was added and the mixture stirred for 5 min on a magnetic stirrer. Then the mixture hexane/acetone/EtOH (2:1:1) was added (30 mL), the whole

mixture first stirred for 5 min and then shaken for 30 min. To enhance the separation of the organic layer, a 5-mL portion of water was added, the mixture stirred for another 5 min, centrifuged for 10 min at 3000 rpm (or left aside for 15 min); the first hexane extract was quantitatively removed afterwards. Then, 15 mL hexane was added to the remaining material, the mixture stirred for 5 min and shaken for 30 min and the second extraction step finished as described above. The latter procedure was repeated two more times (upto four extractions), if needed. Hexane extracts were combined as an equivolume mixture. UV-Vis spectra of combined extracts were recorded in the range 320-600 nm and the absorbance in the absorption peak was measured versus hexane. Signals measured for the samples were corrected by the signal of the real blank; the latter served for calculating the TC concentration.

For low-TC samples (from white corn, wheat, sorghum and some soybeans) 1–2 extractions suffice; for high-TC samples (from yellow corn) 3–4 extractions are needed.

All the experiments were performed under from light protected conditions.

TC was calculated using the absorption coefficient for the mixture of the carotenoids in hexane of $A_{1\text{cm}}^{1\%} = 2500 \text{ dL g}^{-1} \text{ cm}^{-1}$.^{21,17,18} This approach was checked versus three types of calibrations: calibration in hexane, calibration in real blank and the method of standard additions (data not shown).

As the reference method the »Screening method for dry corn« described by Rodriguez-Amaya & Kimura¹⁷ and Kimura et al.¹⁸ was applied; procedure including room temperature rehydration and extraction with mortar and pestle (two 50-mL portions of acetone) combined with partition in petroleum ether was used.

Figures of merit of the new method were evaluated. The method's accuracy was checked versus reference standard material and versus reference method (Table 1). Limit of detection (*LOD*) and lower limit of quantitation (*LLOQ*) were estimated based on real blank data and criteria set by ICH²² (signal-to-noise = 3:1, where noise stands for the standard deviation of the real blank) and Braggio et al.²³, respectively. Precision was expressed as repeatability (within-a-batch precision) and/or intermediate precision (between batches).

3. Results and Discussion

3. 1. Method Refinement

During the process of conditions optimization solvent for hydration, solvent for extraction and respective time conditions were defined.

Hexane/acetone/ethanol (2:1:1) has been preferred over acetone as the extraction solvent due to its extraction efficacy and selectivity. This extraction mixture was introduced by Sadler et al.²⁴ and used by Sharma and le

Table 1. Accuracy data according to reference material and reference method

Certified reference material	TC certified (mg kg ⁻¹) ^a	A _{1cm} ^{1%} -approach (this paper) ^b			
		TC found, mean±SD (mg kg ⁻¹) (n = 6) ^{a,c}	Recovery, mean±SD (%)	e _{max} (±%)	RSD (%) ^h
BCR@-485	71.9	73.4±2.8	102.1±3.9	6	3.8
		Calibration (this paper) ^d			
		72.5±2.8	100.8±3.9	5	3.9
Quality control sample code		A _{1cm} ^{1%} -approach (this paper) ^b		Reference method ^{17,18}	
		TC found, mean±SD (mg kg ⁻¹) ^e	RSD (%) ⁱ	TC found, mean±SD (mg kg ⁻¹) ^e	RSD (%) ^h
QC1	0.9±0.1 (n = 7) ^f	11.7	0.9±0.2 (n = 5)	19.3	
		F = 2.754 [F _{tab} = 4.76 (95)], p = 0.927: HD, NS			
QC2	10.5±0.8 (n = 24) ^g	7.3	10.1±1.0 (n = 3)	9.8	
		F = 1.671 [F _{tab} = 3.33 (95)], p = 0.298: HD, NS			
QC3	17.3±1.0 (n = 28) ^g	5.9	17.4±0.5 (n = 3)	2.8	
		F = 4.319 [F _{tab} = 19.45 (95)], p = 0.891: HD, NS			

QC1 – quality control 1, white corn flour; QC2 – quality control 2, yellow corn flour; QC3 – quality control 3, yellow corn grits; e_{max} – maximal relative error, n – number of independent analyses, SD – standard deviation, RSD – relative standard deviation. Statistical inference: HD – homoscedastic data; NS – non-significant difference between the results.

^a Dry mass basis. λ_{max} at 446 nm. ^b Approach based on A_{1cm}^{1%} for mixture of carotenoids in hexane of 2500 dL g⁻¹ cm⁻¹. ^c Fourfold extraction.

^d Calibration with β-carotene (β-carotene 30% FS; Roche, Basel, Switzerland) in hexane (0.10–1.98 μg mL⁻¹) at 449 nm: y = 0.2472x + 0.0033, R² = 0.9998. ^e Wet mass basis. λ_{max} range: 441–447 nm. ^f Double extractions. ^g Triple/fourfold extractions. ^h Repeatability. ⁱ Intermediate precision.

Maguer,²⁵ Lee and Castle,²⁶ Bicanic et al.,^{27,28} and Luterotti et al.,²⁹ for extractions of carotenes from tomato products, fruit juices and margarines.

3. 2. Spectral Characteristics

Diversity of carotenoids and their isomers is expected in corn flour samples. According to Hulshof et al.,¹⁴ Panfili et al.,¹⁶ Rodriguez-Amaya & Kimura¹⁷ and Kimura et al.¹⁸ dominating carotenoids in corn are provitamin A inactive carotenoids zeaxanthin (30–60%) and lutein (10–40%), followed by those which are vitamin A precursors such as β-carotene (4–30%), β-cryptoxanthin (10–20%) and α-carotene (1–3%). Similarly, in the sorghum grain zeaxanthin is dominating (30–50%), followed by lutein (20–30%) and ca 10% β-carotene.^{11b} In the case of wheat flours about 90% of lutein is followed by ca 5% of zeaxanthin and a few percents of α+β-carotene.¹⁶ The method we are suggesting aims at determination of the concentration of total carotenoids, including all their geometric isomers: carotenes (α- and β-), monohydroxy carotenoids (β- and possibly α-cryptoxanthin, zeinoxanthin), and dihydroxycarotenoids (zeaxanthin and lutein). It should be kept in mind that visible spectra of α-carotene, α-cryptoxanthin/zeinoxanthin and lutein are practically the same; the same applies to β-carotene, β-cryptoxanthin and zeaxanthin.^{17,18} This makes the spectra of their mixtures relatively simple.

Spectra recorded from hexane extracts of samples investigated in this research featured the characteristic

three-peak carotenoid pattern (see Figs. 1a–c). Majority of carotenoids passed into the first hexane extract (70–90%), and about 10–20% into the second hexane extract. The spectral profile observed in the first hexane extract of corn flours and corn grits usually includes peaks at 440–450 and 470–480 nm and shoulders at 420–430 nm and at ca 400 nm (see Fig. 1a). Tocopherols are evident from the strong absorption at 295–300 nm. In the first polar layer separated from yellow corn flours (YCF1 and YCF2) and corn grits (YCG1) a strong absorption is seen between 320 and 340 nm which still needs to be elucidated. In the second hexane extract and in the polar phase shoulder at 420–430 nm usually changed into a peak and the peak at 470 nm changed into a shoulder. This may indicate that the composition of the extracts vary between the extraction steps.

Spectra of the combined hexane extracts of various samples are displayed in Figs. 1b and 1c. Corn flours, even the low-TC ones such as white corn flours, and corn grits show typical carotenoid spectral pattern in hexane. This applies also to some wheat flours and wheat grits, soybean and sorghum flours. Partition of TC from corn products between the hexane and the polar layer (consisting of water, ethanol and acetone) might result in 0–10% TC in the first polar phase. Its spectrum shows weak carotenoid profile which is superimposed onto a strong base line (see Fig. 1a).

The „middle“ carotenoid peak made the basis for quantitation of TC in hexane extract. For yellow corn flours and corn grits λ_{max} lied between 445 and 448 nm,

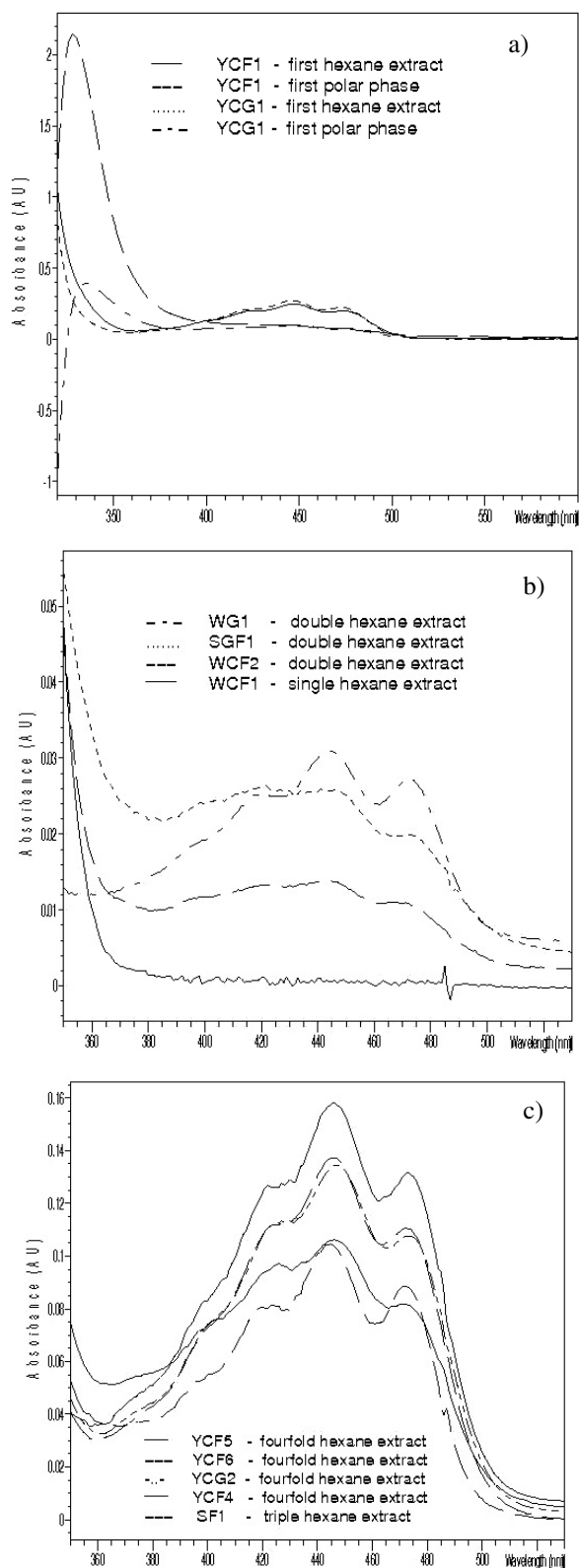


Figure 1: Spectra of: a) first hexane and first polar phase separated from yellow corn flour YCF1 and yellow corn grits YCG1; b) single hexane extract of real blank WCF1, combined double hexane extracts of white corn flour WCF2, sorghum flour SGF1 and wheat grits WG1; c) combined triple hexane extract of soybean flour SF1 and fourfold hexane extracts of yellow corn flours YCF4, YCF5 and YCF6, and yellow corn grits YCG2.

for white corn flours between 441 and 446 nm, for soybean flours between 445 and 446 nm, for wheat flours and grits at 443 nm and for sorghum flour at 446 nm.

3. 3. Extraction Efficiency

The general procedure could be adapted to samples with different TC concentrations using different number of extraction steps, which means that actually two methods have been proposed: the rapid one needing 1 to 2 extractions only for low-TC samples from white corn, wheat, soybean and sorghum, the second one using 3 to 4 extractions for high-TC samples from yellow corn.

Extraction efficiency as a function of number of extraction steps for high-TC yellow corn flour YCF3 and low-TC white corn flour WCF2 is presented in Figs. 2a, b.

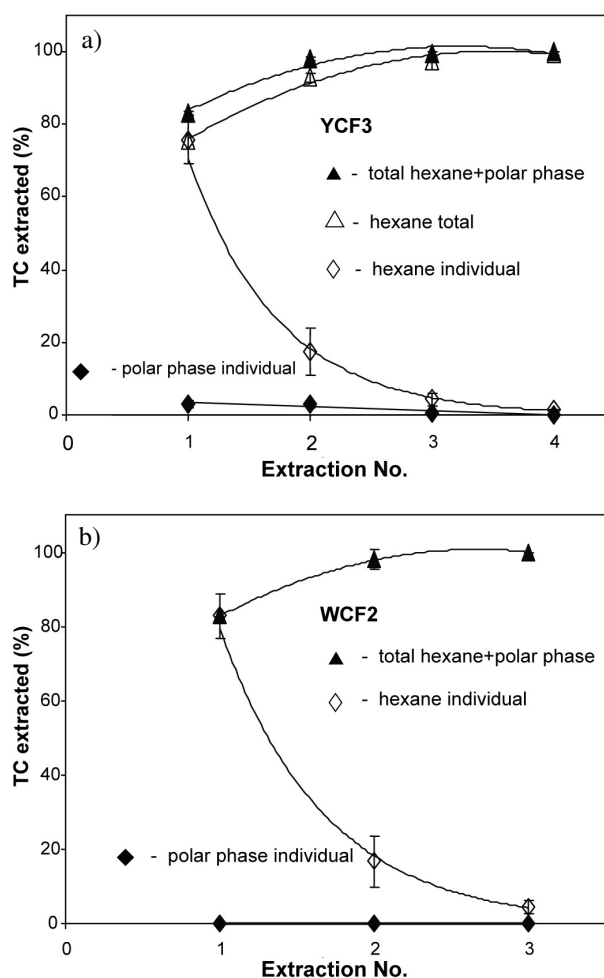


Figure 2: TC extraction efficiency as a function of number of extractions for: a) yellow corn flour (sample YCF3): \diamond – individual values in hexane ($n = 7-8$), \blacklozenge – individual values in polar phase ($n = 2-8$), \triangle – cumulative values in hexane ($n = 8-16$), \blacktriangle – sum of cumulative values for hexane and polar phase ($n = 2-16$); b) white corn flour (sample WCF2): \diamond – individual values in hexane ($n = 3-4$), \blacklozenge – individual values in polar phase ($n = 3-6$), \blacktriangle – sum of cumulative values in hexane and in polar phase ($n = 3-6$) (n – number of independent analyses, SD bars indicated).

As far as high-TC flour and grits samples are concerned the most accurate results are obtained if four successive extractions were carried out (see Figs. 1c and 2a). However, for rapid routine analyses number of extraction steps might be reduced to three but the negative bias of 2.8% should be taken into account. As well, for a rapid screening of white corn samples, one extraction may suffice instead of two.

During extractions TC partitioned between hexane and polar phase. For example, in the case of yellow corn grits and non-milled corn flours 70–90% TC was eliminated into hexane after two extractions; after milling of the flours this amount of TC increased to 90–100%. Similarly, after the third extraction 90–100% TC was found in hexane. Accordingly, the amount that remained in the polar phase decreased down to $\leq 10\%$. This indicated the necessity of milling the coarse samples which could additionally reduce the consumption of solvents and time.

70–100% TC from white corn flours, wheat flours and wheat grits come into the first hexane extract but none could be detected in the respective polar portion. The rest of TC, namely upto 30%, appeared in the second hexane extract. In the case of some soybean flours (SF2) and sorghum flour (SGF1) 60–100% TC might be found in the

first hexane extract and the rest in the second hexane extract and the double extracts were found satisfactory. Some soybean, wheat and even white corn flours may lose all TC in the first hexane layer confirming that even one extraction step might be sufficient. It seems that reduced number of extraction steps (1 to 2) is adequate for low-TC samples, namely those with $< 1.5 \text{ mg kg}^{-1}$ TC (see Figs. 1b and 2b).

3. 4. Analytical Figures of Merit

Accuracy check was performed, using the reference material, lyophilised vegetable mix BCR®, and versus the reference method (Table 1). Recovery of $102.1 \pm 3.9\%$ and repeatability of 3.8% were evaluated for the suggested method using BCR®. Moreover, both, suggested method and the reference method were found not to be statistically different in terms of mean TC concentration found in the samples as well as in terms of precision at 95% probability level.

We found that standard additions and calibration approaches were redundant (data not shown). Linear response was found upto $3 \mu\text{g mL}^{-1}$ TC, accompanied by high coefficient of correlation (> 0.999) and low residual sum of the squares (6.7×10^{-5} to 1.2×10^{-2}).

Table 2. Analyses of yellow and white corn, wheat, soybean and sorghum flours and grits

Sample code/ λ_{max} (nm)	$A_{1\text{cm}}^{1\%}$ -approach (this paper) ^a		Sample code/ λ_{max} (nm)	$A_{1\text{cm}}^{1\%}$ -approach (this paper) ^a	
	TC, mean \pm SD (mg kg^{-1}) ^b	RSD (%)		TC, mean \pm SD (mg kg^{-1}) ^b	RSD (%)
WCF2/ 441–445 ^c	0.9 \pm 0.1	0.1 ($n = 3$) ^e 11.7 ($n = 7$) ^f	YCG2/ 447 ^d	17.3 \pm 1.0	2.5–5.7 ($n = 3$ –5) ^e 5.9 ($n = 28$) ^f
WCF3/ 446 ^c	0.7 \pm 0.2	23.4 ($n = 4$) ^f	YCG3/ 446 ^d	22.1 \pm 0.6	2.8 ($n = 3$) ^e
YCF1/ 448 ^d	22.9 \pm 0.2	0.8 ($n = 3$) ^e	YCG4/ 446 ^d	18.6 \pm 0.3	1.4 ($n = 3$) ^e
YCF2/ 447 ^d	11.7 \pm 1.0	3.7–4.8 ($n = 3$) ^e 8.1 ($n = 10$) ^f	WF1/ 443 ^c	1.1 \pm 0.2	17.9 ($n = 4$) ^f
YCF3/ 447 ^d	14.5 \pm 0.4	0.4–2.3 ($n = 3$) ^e 2.4 ($n = 10$) ^f	WF2/ 443 ^c	1.3 \pm 0.2	12.9 ($n = 4$) ^f
YCF4/ 447 ^d	11.9 \pm 0.9	0.8–3.1 ($n = 3$ –6) ^e 7.3 ($n = 26$) ^f	WG1/ 443 ^c	1.6 \pm 0.2	10.9 ($n = 4$) ^f
YCF5/ 446 ^d	20.9 \pm 1.2	0.4–4.1 ($n = 3$) ^e 5.5 ($n = 20$) ^f	SF1/ 445 ^c	9.9 \pm 1.0	10.5 ($n = 4$) ^f
YCF6/ 446 ^d	18.5 \pm 0.7	1.7–3.4 ($n = 3$ –6) ^e 3.7 ($n = 16$) ^f	SF2/ 443 ^c	0.9 \pm 0.3	34.7 ($n = 4$) ^f
YCF7/ 446 ^d	10.5 \pm 0.8	0.5–9.0 ($n = 3$ –4) ^e 7.3 ($n = 24$) ^f	SGF1/ 446 ^c	1.5 \pm 0.1	5.0 ($n = 4$) ^f
YCG1/ 446 ^d	22.0 \pm 0.2	0.8 ($n = 3$) ^e			

WCF – white corn flour, YCF – yellow corn flour, YCG – yellow corn grits, WF – wheat flour, WG – wheat grits, SF – soybean flour, SGF – sorghum flour, n – number of independent analyses, SD – standard deviation, RSD – relative standard deviation

^a Approach based on $A_{1\text{cm}}^{1\%}$ for mixture of carotenoids in hexane of $2500 \text{ dL g}^{-1} \text{ cm}^{-1}$. ^b Wet mass basis. ^c Double hexane extractions.

^d Triple/quadrupole hexane extractions. ^e Repeatability. ^f Intermediate precision.

LOD and LLOQ estimates found were 0.2 and 0.6 mg kg⁻¹ TC, respectively.

Repeatability and intermediate precision of analyses of yellow corn samples did not exceed 9%: only in the case of low-TC samples (white corn flours, wheat grits and flours, soybean flour) intermediate RSD exceeded 10% (Table 2). Taking into account the complexity of the analyzed samples it follows that the favourable precision has been achieved.

Compared to some existing methods, the method proposed in this manuscript obviates the saponification step and the chromatographic separation,^{e.g.,7} and uses markedly smaller quantity of the sample,^{e.g.,17,18} regardless of TC concentration in the sample or the sample type. On the contrary, carotenoids concentration controls the analysis time making the analysis of low-TC samples, when number of extractions is reduced to only one to two, rapid. The method is applicable to analysis of products made from several crop types.

3. 5. TC Concentration in Flour and Grits Samples

Table 2 presents the concentration data for TC in various samples investigated (in mg kg⁻¹): white corn flours 0.7–0.9, yellow corn flours 11–23, yellow corn grits 17–22, wheat flours 1.1–1.3, wheat grits 1.6, sorghum flour 1.5 and soybean flours 0.9–9.9. Moreover, from the single hexane extraction, concentration of 0.13±0.06 mg TC kg⁻¹ (*n* = 3) was found in real blank WCF1 at 446 nm.

Our data for TC concentration in yellow corn flours are in accordance with the data reported by other authors. Hulshof et al.¹⁴ reported on 9.9 to 40.0 mg kg⁻¹ total carotenoids and Kurilich & Juvik¹³ about 9 mg kg⁻¹ total carotenoids in several maize lines. Kimura et al.¹⁸ found 15–22 mg kg⁻¹ total carotenoids in corn. Moreover, Hentschel et al.¹⁹ found 1.5–4 mg kg⁻¹ *all-trans* lutein in durum wheat accompanied by minor amounts of zeaxanthin.

4. Conclusion

Determination of total carotenoids, including all their geometric isomers was done in cereal grain products: corn flours and grits, soybean and sorghum flours and even wheat flours and grits by the use of absorption coefficient-based spectrophotometric method. The experiments showed that three to four extraction steps were necessary for complete removal of carotenoids into hexane phase in the case of typical yellow corn products but for the samples with low TC concentration such as these made from white corn, wheat, sorghum or even soybean one to two extractions suffice.

The method demonstrates respectable accuracy, precision and sensitivity. The facts that saponification is obviated, that either calibration or standard additions are re-

dundant, together with short-term hydration, result in a simple and cheap analytical method. In the case of low-TC samples it can be very rapid as well.

Important to say, TC value actually reflects on the concentration of total xanthophylls being known of high concentration predominance (>80%) over carotenes in cereal grain products investigated. The proposed method was proven reliable enough for routine analyses of commercial cereal grain products and control of their nutritional value, including newly analysed samples such as white corn flours, yellow corn grits and soybean flours.

5. Acknowledgement

This research is co-financed by the research grants of the Ministry of Science, Education and Sports, Republic of Croatia: «Interactions of gallium(III) and iron(III) with antiproliferative drugs» (006-0061117-1243) and «Nutritional, antioxidative and prebiotic attribute of corn for domestic animals» (178-1780496-0368). The authors thank the Faculty of Agronomy, University of Zagreb, for donating white corn flour and sorghum flour species.

6. References

1. J. L. Groff, S. S. Gropper, S. M. Hunt, in: *Advanced Nutrition and Human Metabolism*, West Publishing Co., New York, NY, USA, **1995**, pp. 284–312.
2. H. Sies, W. Stahl, *Am. J. Clin. Nutr.* **1995**, *62*, 1315s–1321s.
3. A. V. Rao, L. G. Rao, *Pharmacol. Res.* **2007**, *55*, 207–216.
4. L. M. Canfield, N. I. Krinsky, J. A. Olson, Carotenoids in Human Health, in: *Annals of New York Academy of Sciences*, Vol. 691, The New York Academy of Sciences, New York, NY, USA, **1993**.
5. E. J. Johnson, *Nutr. Clin. Care* **2002**, *5*, 47–49.
6. S. Agarwal, A. V. Rao, *Drug Metab. Drug Inter.* **2000**, *17*, 189–210.
7. AOAC, Carotenes and Xanthophylls in Dried Plant Materials and Mixed Feeds. Method 970.64, in: K. Helrich (Ed.): *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc. Arlington, VA, USA, **1990**, pp. 1048–1049.
8. L. Feltl, V. Pacáková, K. Štulík, K. Volka, *Curr. Anal. Chem.* **2005**, *1*, 93–102.
9. D. B. Rodriguez-Amaya, *A Guide to Carotenoid Analysis in Foods*, ILSI Human Nutrition Institute, Washington, DC, USA, **2001**.
10. H. Pfander, R. Riesen, U. Niggli, *Pure Appl. Chem.* **1994**, *66*, 947–954.
11. C. W. Blessin, *Cereal Chem.* **1962**, *39*, a: 236–242; b: 389–392.
12. E. J. Weber, *JAOCs*, **1987**, *64*, 1129–1134.
13. A. C. Kurilich, J. A. Juvik, *J. Agr. Food Chem.* **1999**, *47*, 1948–1955.

14. P. J. M. Hulshof, T. Kosmeijer-Schuil, C. E. West, P. C. H. Hollman, *J. Food Comp. Anal.* **2007**, *20*, 655–661.
15. T. Muzhingi, K. J. Yeum, R. M. Russell, E. J. Johnson, J. Qin, G. Tang, *Int. J. Vitam. Nutr. Res.* **2008**, *78*, 112–120.
16. G. Panfili, A. Fratianni, M. Irano, *J. Agr. Food Chem.* **2004**, *52*, 6373–6377.
17. D. B. Rodriguez-Amaya, M. Kimura, HarvestPlus Handbook for Carotenoid Analysis, HarvestPlus Technical Monograph 2, Washington, DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT), **2004**, <http://www.harvestplus.org/sites/default/files/tech02.pdf> (accessed: November 26, 2010).
18. M. Kimura, C. N. Kobori, D. B. Rodriguez-Amaya, P. Nestel, *Food Chem.* **2007**, *100*, 1734–1746.
19. V. Hentschel, K. Kranl, J. Hollmann, M. G. Lindhauer, V. Böhm, R. Bitsch, *J. Agr. Food Chem.* **2002**, *50*, 6663–6668.
20. Y. Choi, H.-S. Jeong, J. Lee, *Food Chem.* **2007**, *103*, 130–138.
21. B. H. Davies, Carotenoids, in T. W. Goodwin (Ed): Chemistry and Biochemistry of Plant Pigments, Academic Press, London, **1976**, pp. 38–165.
22. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Current Step 4 version, Geneva, November **2005**, <http://www.ich.org/LOB/media/MEDIA417.pdf> (accessed: December 7, 2009).
23. S. Braggio, R. J. Barnaby, P. Grossi, M. Cugola, *J. Pharm. Biomed. Anal.* **1996**, *14*, 375–388.
24. G. Sadler, J. Davis, D. Dezman, *J. Food Sci.* **1990**, *55*, 1460–1461.
25. S. K. Sharma, M. le Maguer, *Ital. J. Food Sci.* **1996**, *2*, 107–113.
26. H. S. Lee, W. S. Castle, *J. Agric. Food Chem.* **2001**, *49*, 877–882.
27. D. Bicanic, J. Swarts, S. Luterotti, G. Pietraperza, O. Doka, H. de Rooij, *Anal. Chem.* **2004**, *76*, 5203–5207.
28. D. Bicanic, J. Swarts, S. Luterotti, P. Helander, V. Fogliano, *J. Agric. Food Chem.* **2005**, *53*, 3295–3299.
29. S. Luterotti, D. Bicanic, R. Požgaj, *Anal. Chim. Acta* **2006**, *573–574*, 466–473.

Povzetek

V mokah in zdrobih žitaric in soje smo z novo validirano spektrometrično metodo določili skupne karotenoide, ki so merilo skupne koncentracije ksantofilov. Analizni postopek je zlahka prilagodljiv različnim vzorcem, število ekstrakcijskih stopenj pa je odvisno od koncentracije karotenoidov v vzorcu. Predlagani sta dve metodi: hitra- za vzorce z nizko koncentracijo karotenoidov (bela koruza, pšenica, soja, sirek), ki vključuje 1–2 ekstrakciji in druga metoda – za vzorce z visoko koncentracijo karotenoidov (rumena koruza), ki potrebujejo 3–4 ekstrakcije. Metoda, ki ne zahteva umeritve s standardnimi raztopinami je relativno hitra, enostavna in poceni.

Točnost metode smo potrdili z analizo standardnega referenčnega materiala, ki je pokazala $102.1 \pm 3.9\%$ referenčne vrednosti in na osnovi primerjave z referenčno metodo. Na podlagi 9 % standardnih odmkov meritev smo potrdili dobro ponovljivost in vmesno natančnost metode, njeno občutljivost pa opredeljujeta spodnja meja detekcije $0,2 \text{ mg TC kg}^{-1}$ in spodnja meja kvantifikacije $0,6 \text{ mg TC kg}^{-1}$.

Metodo smo uspešno uporabili za ugotavljanje prehranske vrednosti izdelkov iz žitaric z vidika skupne koncentracije karotenoidov, ki so se gibale v koncentracijskih območjih $11\text{--}23 \text{ mg kg}^{-1}$ za rumene koruzne moke, $0,7\text{--}0,9 \text{ mg kg}^{-1}$ za bele koruzne moke, $17\text{--}22 \text{ mg kg}^{-1}$ za rumene koruzne zdrobe, $1,1\text{--}1,6 \text{ mg kg}^{-1}$ za pšenične moke in zdrobe, $1,5 \text{ mg kg}^{-1}$ za sirkovo moko in $0,9\text{--}9,9 \text{ mg kg}^{-1}$ za sojine moke.