NOTE

Modification of AACC Procedure for Measuring β-Carotene in Early Generation Durum Wheat

M. Santra,[1–3] V. S. Rao,[1] and S. A. Tamhankar[1]

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Durum wheat (*Triticum turgidum* ssp. *durum*) is highly valued for pasta making in many parts of the world due to its attractive bright appearance which is caused by xanthophylls and carotenoids. Among carotenoids, β-carotene is important for both pasta quality and nutritional value because it acts as a preservative and is a precursor of vitamin A. Durum wheat normally has higher pigment content than nondurum wheat (Dick and Matsuo 1988).

Until now, color evaluation of pasta depended mostly on visual comparisons with standard samples. Ferrari and Bailey (1929) developed a colorimetric method for determination of carotene in flour that used 20 g of wheat in gasoline. A method known as the Pekar test for comparing the color of cereal products was reported by Geddes et al (1934). The accuracy of this test is entirely dependent on the skill and visual acuity of the operator. Goulden et al (1934) studied carotenoid content in a series of hybrid wheat using Approved Method 14-50 (AACC 2000). Markly and Bailey (1935) determined the carotenoid pigment concentration using as little as 0.55 g of wheat flour in a naphtha-alcohol mixture. But this method involved many steps and was not amenable for large number of samples. Binnington et al (1941) standardized the Evelyn photometric colorimeter for flour pigment determination using 20 g of flour in butyl alcohol. Konzak et al (1973) established another rapid microscreening method for semolina color applicable to early generation materials. The samples (2–5 g) were scored visually and then compared with the results obtained by a Neotec Du-Color matching instrument. The AACC Approved Method 14-50, while accurate for spectrophotometric measurement of β-carotene, requires 8 g of flour. None of these published methods are useful for measuring β-carotene in early generations like BC_1, F_1, or F_2 where quantity of flour is limited by the number of seeds available. Therefore, our objective was to develop a method using a small quantity of flour that would be useful for screening early generation material. In this report, we describe a modification of AACC Approved Method 14-50 for determining β-carotene content in as little as 0.125 g of wheat flour.

MATERIALS AND METHODS

Two Indian durum wheat cultivars, PDW-233 and Bhalegaon-4, with high and low β-carotene content, respectively, were selected for the study. The grains were ground uniformly to fine powder in a micromill (Cyclotek, Tecator) with a 0.5-mm sieve. Care was taken to avoid any mixing with previous samples.

The procedure for β-carotene determination used here was based on Approved Method 14-50 (AACC 2000). Water-saturated *n*-butanol (WSB) was prepared by mixing *n*-butanol with distilled water in an 8:2 ratio. Seven sample lots (8–0.125 g of flour) with three replicates were used (Table I). Sample lots of 8 and 4 g were tested in 150-mL glass-stoppered Erlenmeyer flasks; sample lots of 2, 1, 0.5 g were tested in 10-mL capped plastic centrifuge tubes; sample lots of 0.25 and 0.125 g were tested in 1.5-mL microcentrifuge tubes. WSB was added to each of the seven sample lots at 40, 20, 10, 5, 2.5, 1.25 and 0.625 mL, respectively. The flasks and tubes containing flour and WSB were vigorously mixed either by handshaking or by vortexing, and these were kept in the dark for 16–18 hr for extraction of β-carotene. For samples of 8 and 4 g, the upper phase from the flasks was separated using a funnel as described in Approved Method 14-50. For other samples, tubes were spun at 10,000 × *g* for 10 min to recover the supernatant. The absorbance of supernatant was measured at 440 nm on a spectrophotometer (Hitachi U-3210) using microcuvette. A calibration curve was made from known quantities of pure β-carotene (Sigma C-9750). β-Carotene content (ppm) of the samples was derived from the calibration curve. A fresh standard curve was made for every batch of observation.

RESULTS AND DISCUSSION

The β-carotene content of seven sample lots for both cultivars is shown in Table I. Very little variation was observed in the seven lots for either cultivar. Analysis of variance (ANOVA) for both cultivars is shown in Table II. Calculated *F* values showed no significant difference between the β-carotene content estimated from the largest quantity (8 g) and the smallest quantity (0.125 g) of either cultivar.

Because the quantity of β-carotene reduces over time, fresh standard solution was always used to establish the correct standard curve. The β-carotene content is also reduced slowly due to lipoxygenase enzyme activity (Ferrington et al 1981), so estimation was done immediately after grinding without any flour storage.

TABLE I
β-Carotene Content (ppm) in Two Indian Durum Wheat Cultivars

Sample (wt. g)	Reagent (mL)	No.of Samples (×3)	Cultivar[a] PDW-233	Cultivar[a] Bhalegaon-4
8	40	1	7.71 ± 0.015	3.14 ± 0.060
4	20	2	7.60 ± 0.028	3.26 ± 0.001
2	10	4	7.79 ± 0.115	3.40 ± 0.037
1	5	8	7.65 ± 0.044	3.21 ± 0.014
0.5	2.5	16	7.89 ± 0.045	3.43 ± 0.027
0.25	1.25	32	7.79 ± 0.012	2.94 ± 0.032
0.125	0.625	64	7.77 ± 0.001	3.27 ± 0.007

[a] Mean and standard deviation (SD).

TABLE II
ANOVA for PDW-233 and Bhalegaon-4

Source	df	SS	MS	*F*
PDW-233				
Between samples	6	0.17	0.028	5.5
Within samples	2	0.01	0.005	
Bhalegaon-4				
Between samples	6	0.19	0.032	16
Within samples	2	0.004	0.002	

[1] Genetics and Plant Breeding Group, Plant Sciences Division, Agharkar Research Institute, Pune 411008, India.

[2] Corresponding author. Phone: 515-294-2062. Fax: 515-294-6505. E-mail msantra@iastate.edu.

[3] Present address: Department of Agronomy, Iowa State University, Ames, IA 50011.

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This modified method has several advantages over Approved Method 14-50. It uses only 0.125 g of flour, which is 64× less than the quantity used in the original AACC method. It is simple and reproducible. Microcentrifuge tubes can be handled easily and require less time and space than the 150-mL flasks described in the original AACC method. So a large number of samples can be estimated easily at one time using this method. During stirring in a 150-mL flask, some of the 8 g of flour adheres to the sides and cannot be brought easily to the solution. Using microcentrifuge tubes, the stirring step can be omitted because flour can be mixed simply by vortexing and precipitated by spinning, thus avoiding the filtration.

Because this method requires very little flour, it can be applied in durum wheat breeding for screening early generation breeding lines like BC_1, F_1, or F_2 where seed material, and thereby flour quantity, is limited.

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