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Selected nutrient analyses of fresh, fresh-stored, and frozen fruits and vegetables

Linshan Li^a, Ronald B. Pegg^{a,*}, Ronald R. Eitenmiller^a, Ji-Yeon Chun^b, Adrian L. Kerrihard^c^a Department of Food Science & Technology, College of Agricultural and Environmental Sciences, The University of Georgia, 100 Cedar Street, Athens, GA, 30602, USA^b Department of Food Science and Technology, Suncheon National University, 413 Jungangro, Suncheon, Jeonnam, 540-742, South Korea^c Department of Nutrition and Food Studies, College of Education and Human Services, Montclair State University, 1 Normal Ave., Montclair, NJ, 07043, USA

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ABSTRACT

This two-year study compared the status of targeted nutrients in selected fresh and frozen fruits and vegetables. In addition, a novel third category was examined—a “fresh-stored” categorization intended to mimic typical consumer storage patterns of produce following purchase (five days of refrigeration). Broccoli, cauliflower, corn, green beans, green peas, spinach, blueberries, and strawberries of all three categories of freshness were analyzed for their concentrations of L-ascorbic acid (vitamin C), *trans*-β-carotene (provitamin A), and total folate. Analyses were performed in triplicate on representative samples using standardized analytical methods and included a quality control plan for each nutrient. In the majority of comparisons between nutrients within the categories of fresh, frozen, and “fresh-stored”, the findings showed no significant differences in assessed vitamin contents. In the cases of significant differences, frozen produce outperformed “fresh-stored” more frequently than “fresh-stored” outperformed frozen. When considering the refrigerated storage to which consumers may expose their fresh produce prior to consumption, the findings of this study do not support the common belief of consumers that fresh food has significantly greater nutritional value than its frozen counterpart.

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1. Introduction

There is strong evidence that public health could be improved by increased consumption rates of fruits and vegetables (CDC, 2013). Many fruits and vegetables are important sources of nutrients that are consumed at inadequate levels in the U.S., including vitamin A, vitamin C, calcium, magnesium, and others (Agarwal et al., 2015). Fruits and vegetables also frequently contain high concentrations of bioactive compounds, and have been shown to exhibit high antioxidant potentials (Liu, 2013). Furthermore, when prepared without added fats or sugars, fruits and vegetables are generally relatively low in calories, high in dietary fiber, and beneficial to satiety (Fulton et al., 2016). The consumption of fruits and vegetables has been shown to aid in healthy weight maintenance, and associate with a reduced risk of multiple chronic diseases (CDC, 2013).

Despite these points, a 2013 Center for Disease Control (CDC) reports that 33% of American adults consume less than one serving of fruits and vegetables a day. Governmental and public health agencies continue to apply ongoing efforts to improve consumption rates of fruits and vegetables for the benefit of public health. For example, the 2015–2020 USDA Dietary Guidelines for Americans advise individuals to increase their intake of fruits and vegetables to help control total caloric intake and manage body weight (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015; U.S. Department of Agriculture, Agricultural Research Service, 2015). The formal suggestion of MyPlate, the revised USDA Food Pyramid, suggests that half of the plate should be comprised of nutrient-dense foods such as fruits and vegetables. These guidelines also highlight the importance of variety, which is necessary to give the human body the large array of vitamins, minerals and macronutrients it needs.

The disparity between dietary recommendations and noted large-scale dietary patterns is a source of ongoing investigation, and it is apparent the causes are multi-faceted and diverse (Deliens et al., 2014; Haynes-Maslow et al., 2013; Stok et al., 2014). One

* Corresponding author.

E-mail address: rpegg@uga.edu (R.B. Pegg).

documented explanation for inadequate fruit and vegetable consumption is a lack of high quality fresh produce choices for consumers, which may frequently be limited by spoilage and losses during transportation and/or storage (Buzby et al., 2014). This is especially the case during winter months, when quality is generally diminished and cost is often higher. Even when produce remains sufficiently unspoiled so as to merit purchase and consumption, there may be more minor degradations (e.g. enzymatic degradations, cellular respiration and oxidation) that can negatively affect their nutritional benefit (Bouzari et al., 2015).

Prior studies investigating fresh produce have determined fresh produce is frequently picked before peak ripeness, packaged, stored, transported, and then stored again (Blackburn and Scudder, 2009). It has been established in prior investigations that post-harvest exposure to periods of storage and transportation at temperatures above freezing can negatively affect nutrient quality, specifically nutrients with antioxidant potential (Villa-Rodriguez et al., 2015). Nutrients from produce will also be affected by genetic factors, climatic factors, and has been shown to be negatively associated with periods of exposure to light and/or oxygen (Alvarez-Suarez et al., 2014).

In efforts to lessen spoilage and degradation, a number of storage and production techniques have been investigated and introduced for produce in recent years. Prominent among these has been the implementation of modified atmospheres, which has been demonstrated to reduce degradation during storage (Oliveira et al., 2015). However, benefits of modified atmospheres discontinue once the package has been opened at the home of the consumer. Other recent innovations have included investigations into the use of edible oil coatings, ethylene absorbers, and the incorporation of anti-microbial agents into packaging materials (Brandwein et al., 2016; Patrignani et al., 2015; Sahu et al., 2016).

In principle, the freezing of fruits and vegetables could serve to provide a highly beneficial mitigation of the problems of spoilage and/or degradation, and provide consumers increased access to nutritious fruits and vegetables. This outcome has, of course, occurred for many consumers, but evidence shows that the scope of its reach continues to be limited by a persistent public perception that the preservation or processing of fruits and vegetables substantially diminishes nutritional quality (Ares et al., 2014). It has been shown that these perceptions continue to influence the choices of consumers (Haynes-Maslow et al., 2013). The validity of the continuing perception of frozen produce being of relatively lesser nutritional quality is a point worthy of investigation, for if it is in fact not accurate, its persistence may play a negative role in public health.

When comparing the nutritional qualities of fruits and vegetables from different processes (specifically the comparison of fresh produce and frozen produce), a point of relatively infrequent examination is that fresh produce may often remain in the consumer's home for a number of days prior to consumption. According to the research of the Food Marketing Institute, the average number of trips to the supermarket in the United States was 1.5 times per week in 2015 (Food Marketing Institute, 2015). This suggests the average consumer stores purchased food in their home for a period of time of nearly five days prior to a return to the supermarket. This in-house storage may very plausibly contribute to losses of nutritional quality, therefore making the comparison of fresh and frozen produce more complicated than is commonly considered in studies. To the knowledge of the authors, the nutritional quality of fresh produce specifically after a period of refrigerated storage that is intended to replicate consumer storage patterns has not received prior investigation.

The aim of this study was to determine and compare the status of targeted nutrients in selected fresh, frozen, and "fresh-stored" fruits and vegetables. The "fresh-stored" storage parameter (five

days of refrigerated storage) was developed by the researchers for the purpose of approximating typical consumer storage patterns (designed with reference to the data of Food Marketing Institute 2015). The study assessed L-ascorbic acid (vitamin C), *trans*- β -carotene (provitamin A), and total folate concentrations within blueberries, strawberries, broccoli, cauliflower, corn, green beans, spinach, and green peas. The decision of what fruits and vegetables to investigate was predetermined by the Frozen Food Foundation (FFF), the funding agency for this study. Their pick of what to examine was based on the findings reported in a white paper commissioned by the FFF. This document, entitled 'Nutritional comparison of frozen and non-frozen fruits and vegetables: Literature review' was prepared by scientists from the Food Processing Center at the University of Nebraska-Lincoln (Kyureghian et al., 2010). So, the choice of produce and nutrients to be analyzed was not random. The choices were based on U.S. consumption patterns and nutrients (i.e., vitamins C, A, folate, and minerals) stipulated by the FFF as being important

2. Materials and methods

2.1. Materials

Produce, both fresh and private-label frozen, was purchased from supermarkets within a 40 km radius of Athens, GA, USA (i.e., Walmart, Sam's Club, Kroger, Publix, Piggly-Wiggly, Ingles, and Bells). The produce included six vegetables and two fruits, namely broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* var. *botrytis*), sweet corn (*Zea mays* L. convar. *saccharata* Körn), green beans (*Phaseolus vulgaris* L.), green peas (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.), blueberries (*Vaccinium corymbosum* L.), and strawberries (*Fragaria* \times *ananassa*).

ACS-grade meta-phosphoric acid pellets, USP-grade L-ascorbic acid (purity, 99.9%), BD Difco™ *Lactobacilli* broth, *Lactobacilli* agar, folic acid casei medium powder, and Pronase® protease (Cat No. 537002-50KU) were purchased from VWR International (Suwanee, GA, USA). ACS-grade glacial acetic acid, hydrochloric acid, sodium hydroxide, 95% (v/v) ethanol, and toluene as well as 2,6-dichloroindophenol sodium salt hydrate (purity, 98+%), HPLC-grade methanol, HPLC-grade methyl *tert*-butyl ether (MTBE), and pyrogallol were obtained from the Fisher Scientific Company (Suwanee, GA, USA). *trans*- β -Carotene (type I, synthetic, \geq 93%), 1,4- α -D-glucan glucanohydrolase (i.e., α -amylase) from *Aspergillus oryzae* (Cat. No. 10065-50G) and USP-grade folic acid (purity, 99.9%) were procured from the Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Unpurified, but acetone-washed, conjugase was isolated from freshly-slaughtered chicken pancreata acquired from the University of Georgia's Department of Poultry Science (Athens, GA, USA).

Certified reference materials (CRMs) from the European Commission Joint Research Center, Institute for Reference Materials and Measurements, were purchased from the Resource Technology Corporation (Laramie, WY, USA); these included BCR®-431 (Brussels sprouts powder with a certified value of 4.83 ± 0.24 g/kg for vitamin C) and BCR®-485 (mixed vegetables with certified values of 23.7 ± 1.5 mg/kg for *trans*- β -carotene and 3.15 ± 0.28 mg/kg for total folate). Gold Medal, enriched, AP flour – an in-house quality control (QC) marker for the folate assay – was purchased from Kroger (Athens, GA, USA).

2.2. Sample acquisition, storage and preparation

The analyses of this study were performed over the span of two years in six distinct time frames: (1) Summer to Fall Year 1, (2) Fall to Winter Year 1, (3) Winter to Spring Year 1, (4) Summer to Fall Year 2, (5) Fall to Winter Year 2, and (6) Winter to Spring Year 2. The

process of acquiring, storing, and preparing the produce was repeated on the first day of each analysis period.

On the first day of each analysis period, a fresh fruit or vegetable of each food type was procured from six of the supermarkets listed in the “Materials” section. In most cases, Bells was the “backup” store for sourcing a fruit or vegetable (i.e., used as a source only if a produce product was unavailable at one of the other six possible sources). For the purpose of maintaining our sampling procedure as representative of consumer shopping habits, the period of produce in-store storage time prior to purchase was not a controlled factor. Each of the samples were equally divided into two parts: One half was labeled ‘fresh-stored’ and placed in a standard kitchen refrigerator (4 °C) to be stored for 5 days, and the other half was designated ‘fresh’ for exposure to nutrient analysis that same day.

Also on the first day of each analysis period, frozen produce was purchased from the frozen sections of six of the above listed supermarkets. These samples were placed in frozen storage (−20 °C) until analysis.

Prior to analysis, composite samples were prepared by combining a ~200-g portion of produce from each of the six supermarkets (within their designated category of fresh, fresh-stored, and frozen) in a plastic tub (62 × 39 × 24 cm) and mixing well. The compositing, a common practice in food compositional studies, was for the purpose of minimizing the influence of outlier behavior from individual variations within sample types. If required, a representative vegetable sample was removed from the composite for blanching just prior to analysis. The blanching protocol was identical for each vegetable (i.e., 1 min contact in boiling water, transfer to an ice water bath for 3 min for quick cooling, remove excess moisture from the sample by tapping over paper towels). For fresh corn-on-the-cob, kernels were cut from the cob post blanching to prevent enzymatic degradation. All fresh and fresh-stored vegetables were blanched for *trans*-β-carotene analyses. Corn-on-the-cob was the only sample blanched for L-ascorbic acid and folate analyses. In all cases, composite samples were never physically ground until just prior to initiating a nutrient analysis.

2.3. Moisture correction

Moisture analyses were performed gravimetrically, in triplicate, per AOAC Official Method 984.25 and the conversion of nutrients based on blanched weight (b.w.) to fresh weight (f.w.) was calculated using the following equation:

$$\text{Nutrient content (f.w., } \mu\text{g/100 g)} = \text{Nutrient content (b.w., } \mu\text{g/100 g)} \times (1 - M_b)/(1 - M_f)$$

where, M_b is the moisture content of the blanched sample; and M_f is the moisture content of the fresh (unblanched) sample.

2.4. L-Ascorbic acid analysis

L-Ascorbic acid analysis was performed as described in AOAC Official Method 967.21 (AOAC International, 2006a, 2006b; AOAC, 1998). The L-ascorbic acid content was calculated according to the following equation:

$$\text{mg L-ascorbic acid/g} = (X - B) \times (F/E) \times (V/Y)$$

where, X is the average mL for test sample titration; B is the average mL for blank titration; F is the mg of L-ascorbic acid equivalent to 1.0-mL indophenol standard solution; E is the mass (g) of sample assayed; V is the volume of the initial test solution; and Y is the volume of the test solution titrated.

L-Ascorbic acid measurements in fresh and fresh-stored corn-on-the-cob were corrected according to their moisture contents before and after blanching. Each sample was analyzed in triplicate.

2.5. Trans-β-carotene analysis

Samples were saponified and extracted per European Standard Methods EN 12823-1 and -2 (2000). Briefly, 100 mL of 95% (v/v) ethanol and 20 mL of 60% (w/v) KOH were added to 10 g of the finely ground sample, with 1 g of pyrogallol to serve as an antioxidant, and saponified under a blanket of nitrogen in an 81 ± 2 °C water bath 45 min, then removed and placed in an ice water bath. Carotenoids were extracted with a hexane solvent extraction, concentrated to dryness, and quantitatively resuspended in an appropriate volume of mobile phase. HPLC analysis was accomplished via an unpublished method by Sanders, described in a technical note obtained from YMC™ Separation Technologies, entitled “Separation of carotenoids found in algae (YMC™ Separation Technology, 2007)”. An Agilent 1200 series quaternary pump with degasser, autosampler, diode array detector and ChemStation software were employed and fitted with a YMC™ C30 carotenoid HPLC column (4.6 × 250 mm, 3-μm particle size; Waters Corporation, Milford, MA, USA), YMC™ carotenoid S-3 DC guard cartridge (4.0 × 200 mm) and YMC™ direct connect end fitting. Elution parameters entailed an isocratic system at a flow rate of 2.0 mL/min with 75:25 (v/v) CH₃OH:MTBE as the mobile phase. Injection volumes were sample dependent and typically 20 or 100 μL. Visible detection was monitored at $\lambda = 450$ nm and the column temperature was maintained at 35 °C.

The mass concentration, ρ , of total *trans*-β-carotene in mg/100 g of the sample (triplicate samples, averaged) was calculated using the following equation:

$$\rho = (A_s \times C \times V_s \times V_{st} \times 100) / (A_{st} \times M \times V_{is} \times 1000)$$

where, A_s are the peak areas for the β-carotene isomers obtained with the sample test solution; C is the corrected purity of the standard solution (μg/mL); V_s is the total volume of sample test solution (mL); V_{st} is the injection volume of the standard solution (μL); A_{st} is the peak area for *trans*-β-carotene obtained with the standard solution in units of area; M is the sample mass (g); V_{is} is the injection volume of the sample test solution (μL); 1000 is the conversion factor for μg to mg; and 100 is the conversion factor for the content to be reported per 100 g sample.

trans-β-Carotene measurements in fresh and fresh-stored produce were corrected according to their moisture contents before and after blanching. Each sample was analyzed in triplicate.

2.6. Folate analysis

Folate analysis was performed according to the operating procedure for microplate assay of folic acid and total folate with calculation of dietary folate equivalents according to an in-house unpublished report based on AOAC Official Method 2004.05. As summarized below, the procedure includes a trienzyme extraction assay and microplate assay.

2.6.1. Isolating γ-glutamyl hydrolase (conjugase) from chicken pancreata

Difco Laboratories (Sparks, MD, USA) no longer provides its chicken pancreas enzyme; hence, chicken pancreata (~60) were harvested from freshly-slaughtered chickens at the University of Georgia’s Department of Poultry Science farm (Athens, GA, USA). The pancreata were packaged in zip lock pouches and frozen at −80 °C overnight. Then ~15 g of frozen pancreata were combined with ~300 mL of cold acetone (−78 °C, prepared by dry ice addition

to the organic solvent) in a 500-mL Erlenmeyer flask. The contents were blended at 25,000 rpm for 60 s using a PT 3100 Polytron™ homogenizer with a Kinematica PT-DA 3012/2TM generator (Brinkmann Instruments, Westbury, NY, USA). The slurry was poured into a Büchner funnel, lined with Whatman No. 1 filter paper, connected to a suction flask and a vacuum system. After suction filtration, the filter cake was rinsed with $3 \times \sim 100$ mL portions of cold acetone. The crude preparation containing the conjugase enzyme was scraped from the filter paper, allowed to dry in a fume hood at room temperature for 1 h, and then lyophilized in a FreeZone® 2.5-L bench-top freeze dryer (Labconco Corporation, Kansas City, MO, USA) to ensure that all traces of acetone and moisture were removed. The product (referred to as the conjugase powder) was transferred to an amber-glass bottle and stored in a refrigerator at 4 °C until used. Activity of the conjugase was confirmed by running the microbiological assay (details below) using a pteroyltetra- γ -L-glutamic acid standard (Cat. No. 16.254, Schricks Laboratories, Jona, Switzerland). When needed, the lyophilized powder (100 mg) was weighed out, dissolved in 20 mL of the 0.1 M phosphate buffer (pH = 7.8) and sonicated for 5 min. The chicken pancreas conjugase reagent was then filtered through glass wool to remove any particulates.

2.6.2. The trienzyme extraction assay

For each fruit and vegetable, 100 g subsamples were taken from the homogeneous composite and ground in a 70-W Black & Decker one-touch chopper (Model HC306, Applica Consumer Products, Inc., Miramar, FL, USA) just preceding the assay. Triplicate samples were subjected to the trienzyme assay using the following method (NB, the description forthwith is made for a single tube): the ground sample (1.0 g) was weighed into a borosilicate glass tube (36 i.d. \times 155 mm), to which 20 mL of a 0.1 M phosphate buffer (pH = 7.8) containing 1% (w/v) L-ascorbic acid were added and a sufficient quantity of deionized water to bring the volume up to 50 mL. The sample was homogenized with the Polytron homogenizer at 25,000 rpm for 60 s. After homogenization, two drops of toluene were added to retard bacterial activity. The tube was then wrapped with aluminum foil and placed in a Thelco boiling water bath (Model 83, Precision Scientific Co., Coimbatore, India) for 15 min. After this period, the tube and its contents were cooled to room temperature using an ice water bath. Pronase® (1 mL of a 2 mg/mL solution) was added and the tube placed in a 5.0-ft³ Isotemp™ standard lab incubator (Model 650D, Fisher Scientific) at 37 °C for 3 h. The tube was returned to the boiling water bath for 3 min to inactivate the protease, followed by a cooling step. Afterwards, 1 mL of a 20 mg/mL α -amylase solution was added followed by a 2-h period in the incubator at 37 °C. Finally, 4 mL of the chicken pancreas conjugase reagent were added followed by an additional incubation at 37 °C for 16 h. The folate tube was returned to the boiling water bath for 3 min to inactivate the conjugase. Using an ice water bath, the tube was cooled to room temperature and the pH of the solution was adjusted to 4.5 with (1 + 1, v/v) HCl and a pH meter. The acidified solution was then quantitatively transferred to a 100-mL volumetric flask. The flask was filled to mark with deionized water and the solution filtered through Whatman No. 1 filter paper into an Erlenmeyer flask. The extract was dispensed into 1.5-mL disposable/conical microcentrifuge tubes (Cat. No. 20170-038, VWR International), wrapped with aluminum foil, and stored at -40 °C until analyzed.

As an internal QC measure, the folic acid fortified Gold Medal, enriched, all-purpose flour (Kroger, Athens, GA, UGA) was extracted by the trienzyme assay described above. From the nutrition label, 30 g of this flour contains 10% of the recommended daily value of 400 μ g of folic acid. Therefore, the folic acid concentration in flour is roughly 133 μ g/100 g flour.

2.6.3. Microplate assay reagents

2.6.3.1. Lactobacilli broth. BD Difco™ Lactobacilli broth AOAC (15.2 g, Cat. No. 290110, VWR International) was weighed, transferred to a 500-mL Erlenmeyer flask and dissolved in 400 mL of deionized water. Using a hot plate/stirrer, the broth was brought to a boil, held there for 2–3 min, cooled to room temperature using an ice water bath, and then brought to mark. Aliquots of the broth (10 mL) were pipetted into 20 \times 150-mm screw-cap culture tubes (Fisher Scientific). The tubes were loosely capped and autoclaved with a steam sterilizer autoclave (AMSCO, Erie, PA, USA) at 121 °C for 15 min. After the tubes had cooled, they were tightly capped and stored in a 4 °C refrigerator until used.

2.6.3.2. Lactobacilli agar. BD Difco™ Lactobacilli agar AOAC (19.2 g, Cat. No. 290010, VWR International) was weighed, transferred to a 500-mL Erlenmeyer flask and dissolved in 400 mL of deionized water. The same procedure was then followed as described for the preparation of the Lactobacilli broth AOAC except that during cooling, the tubes were positioned on their side to create a slanted angle (i.e., the product is referred to as slants). After the agar had solidified, the tubes were tightly capped and stored at 4 °C refrigerator until used.

2.6.3.3. Lactobacilli culture activation and maintenance. Freeze-dried Lactobacillus casei subsp. rhamnosus (ATCC® 7469™) was acquired from the American Type Culture Collection (Manassas, VA, USA). The lyophilized culture was dispersed in 10 mL of the Lactobacilli broth AOAC (solution A). Between 0.5 to 1 mL of solution A was diluted with another 10-mL portion of Lactobacilli broth AOAC (solution B). Solutions A and B were placed in the incubator at 37 °C for 18 h. If the culture grew well in solution B, it was transferred and streaked onto one of the Lactobacilli agar slants, but if not, then solution A was streaked onto the agar slant. The freshly inoculated slant was incubated at 37 °C for 24 h and then placed in the refrigerator. To maintain viability of the culture, a portion of the culture was taken every 7 d from the stored slant and transferred to a new slant. The new slant was incubated at 37 °C for 24 h and then placed in the refrigerator.

2.6.3.4. Depletion medium. Lactobacilli broth AOAC (7.6 g) and BD Difco™ folic acid casei medium powder (18.8 g, Cat. No. 282210, VWR International) were weighed, transferred to a 500-mL Erlenmeyer flask and dissolved in 400 mL of deionized water. The same procedure was then followed as described for the preparation of the Lactobacilli broth AOAC.

2.6.3.5. Folic acid casei medium. BD Difco™ folic acid casei medium powder (28.2 g) was weighed, transferred to a 500-mL Erlenmeyer flask and dissolved in 300 mL of deionized water. Using a hot plate/stirrer, the media was brought to a boil, held for 1–2 min, cooled to room temperature using an ice water bath, and filled to mark. Prior to filtering, the workbench was wiped with 70% (v/v) ethanol. The medium was then filtered near a flame to help prevent bacterial contamination using a 250-mL Corning™ disposable sterile filter system (Cat. No. 09-761-140, Fisher Scientific). Briefly, the filter system was removed from its aseptic packaging and the spout connected to a vacuum hose. Media was poured into the upper chamber and a vacuum was applied. After all of the solution had been filtered through the 0.22- μ m cellulose acetate membrane, the upper unit of the filter was removed and the bottle then capped tightly. The sterile media was stored in a 4 °C refrigerator, but warmed to room temperature before use in the microplate assay.

2.6.3.6. Folic acid stock solution. Under yellow lighting, USP-grade folic acid (20 mg, CAS 59-30-3, Sigma-Aldrich) was accurately weighed and transferred to an Erlenmeyer flask with ~20 mL of 95% (v/v) ethanol. The solution was then diluted with ~30 mL of deionized water. To facilitate the dissolution of folic acid, the pH was raised to 10.0 using 0.1 N NaOH and then adjusted to 7.0 with 0.05 N HCl. The 50-mL solution was quantitatively transferred to a Pyrex® 100-mL Class A low-actinic volumetric flask and then filled to mark with deionized water. Aliquots (10 mL) of this stock solution were transferred to borosilicate glass culture tubes, which were capped, wrapped with aluminum foil, and stored in a 4 °C refrigerator until used.

2.6.3.7. L-Ascorbic acid reagent. L-Ascorbic acid (1 g, USP grade, CAS 50-81-7, VWR International) was weighed and quantitatively transferred into a 10-mL Class A low-actinic volumetric flask using deionized water. The flask was then diluted to mark and warmed under running water to fully dissolve the crystals. Once dissolved, the solution was re-cooled to room temperature.

2.6.4. Microplate assay

The preparation for the microplate assay was as follows: A tube containing the depletion media was warmed to room temperature. The culture from a 3–5 day old slant was transferred to the depletion media. The tube was then incubated at 37 °C for 6 h before further use. To ensure sterility, all supplies were autoclaved at 121 °C for 15 min and were cooled, capped and/or sealed. These included a 250-mL Erlenmeyer flask filled with 100 mL of deionized water, a 50-mL graduated cylinder, a 125-mL Erlenmeyer flask, Gilson diamond pipette tips, and a jar of 1.5-mL disposable/conical microcentrifuge tubes.

Fruit and vegetable sample extracts (from the trienzyme extraction) as well as those of the wheat flour for QC determinations were thawed to room temperature, and then transferred to 20-mL Wheaton amber vials. The vials were diluted to the appropriate ratio (determined by trial and error; details to follow) with deionized water, and capped. The dilution factor employed was based on the estimated folate concentration for the fruit or vegetable in question. Turbidity was determined for each test sample and was compared to that of the folic acid standard. If the measurements were similar, then the appropriate dilution had been employed in the microbiological assay. If not then a more dilute or concentrated test sample was used. A ratio of 1:10 (v/v) was determined for the QC sample, while a 1:1 ratio was chosen for test samples with a folate range of 0 to 30 µg/100 g fruit or vegetable; 1:3 for test samples with a folate range of 30 to 80 µg/100 g fruit or vegetable; 1:5 for test samples with a folate range of 80 to 150 µg/100 g fruit or vegetable; and 1:10 for test samples with a folate content of 150 to 200 µg/100 g fruit or vegetable. The loosely capped amber vials were autoclaved at 121 °C for 5 min and after they had cooled to room temperature using an ice water bath, the vials were capped tightly.

A working solution of folic acid (~2 ng/mL) was prepared: a tube of the folic acid stock solution (~200 µg/mL) was removed from the refrigerator and tempered to room temperature. A 100-µL aliquot was transferred via a Gilson Pipetman Neo® to a Pyrex® 100-mL Class A low-actinic volumetric flask, diluted to mark with deionized water and mixed well. Once mixed, a 1000-µL aliquot of the first diluted standard was transferred via a Gilson Pipetman Neo® to a second 100-mL Class A low-actinic volumetric flask; the flask was diluted to mark and mixed well.

Before performing the microplate assay, the biosafety, laminar-flow hood (Contamination Control, Inc., Kulpville, PA, USA) was wiped down with 70% (v/v) ethanol and run for >60 min to ensure a sterilized environment in the hood before plating. Under the hood, the folic acid working solution and L-ascorbic acid reagent were

syringe filtered using BD 3-mL Luer-Lok™ syringes (Cat. No. BD309657, VWR International) and 0.22-µm polyvinylidene fluoride (PVDF) sterile filters (Cat. No. 09-720-3, Fisher Scientific) into sterilized 1.5-mL disposable/conical microcentrifuge tubes.

A sterile, 96-well, polystyrene Falcon™ tissue culture plate with a flat-bottom and lid (Cat No. 08-772-2C, Fisher Scientific) was removed from its aseptic package. Using a 12-channel Gilson Pipetman M Multichannel, 300 µL of sterilized water were taken up from a 50-mL Costar™ sterile disposable reagent reservoir (Cat. No. 4871, VWR International) and dispensed along row H (i.e., the bottom row, see Fig. 1 for plate set up) of the microplate as a “blank” row; 150 µL were added to all other rows. Then, 150 µL of the sterile-filtered folic acid working solution (~2 ng/mL), sterilized flour extract, and sterilized fruit and vegetable sample extracts were added to wells G1–G3, G4–G6, and G7–G12, respectively, via a 200-µL single channel Gilson Pipetman. Using the 12-channel Pipetman with sterilized tips, the solutions in row G were mixed 3 ×. After mixing, 150 µL of the solutions (be it folic acid standard, flour extract, or two test sample extracts) were transferred from wells G1–G12 to F1–F12. The solutions in wells F1–F12 were mixed 3 × and 150 µL of the diluted solutions were then transferred to each subsequent row of wells (E thru A), until the last row (A) on the microplate was filled. In row A (wells A1–A12), 150 µL were removed from the microplate and disposed. Growth media for each plate was prepared by adding 15 mL of the folic acid casei medium reagent, 150 µL of the sterilized L-ascorbic acid reagent, and 45 µL of the depletion medium incubated with the culture to a 125-mL sterilized flask, and mixing well. This solution was poured into a sterile reagent reservoir; 150 µL were pipetted into every row, except for the blank row (H). Each prepared microplate was individually placed inside a zip-lock sandwich bag and transferred to the incubator for 18 h at 37 °C.

After 18 h the microplates were removed from the incubator and the contents in the wells of rows A thru G were mixed well with the 12-channel Pipetman. Absorbance readings for the microplate were taken at λ = 650 nm using a FLUOstar Omega microplate reader (BMG LABTECH Inc., Cary, NC, USA). The assay was carried out using bottom scanning and at an incubation temperature of 25 °C.

2.6.4.1. Calculation of folate levels in fruits and vegetables. Turbidity data was collected and concentrations of folate (i.e., folic acid equivalents) in the samples calculated using the microplate reader's MARS Data Analysis software. Polynomial regression (a form of linear regression) was employed to calculate a standard curve based on the absorbance readings of the folic acid standard at different concentrations. For each microplate analyzed, a new standard curve was created; folic acid concentration versus absorbance was fitted with a third-order polynomial. The resultant curve is sigmoidal, but has a linear portion associated with it where concentration can be determined from absorbance readings according to Beer's Law (see Fig. 2). Using this region of

	1	2	3	4	5	6	7	8	9	10	11	12
A	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9	A-10	A-11	A-12
B	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10	B-11	B-12
C	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12
D	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	D-11	D-12
E	E-1	E-2	E-3	E-4	E-5	E-6	E-7	E-8	E-9	E-10	E-11	E-12
F	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11	F-12
G	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	G-12
H	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-9	H-10	H-11	H-12

Folic acid standard
Flour sample for QC
Test sample 1
Test sample 2

Fig. 1. Layout of the microplate for the folate assay.

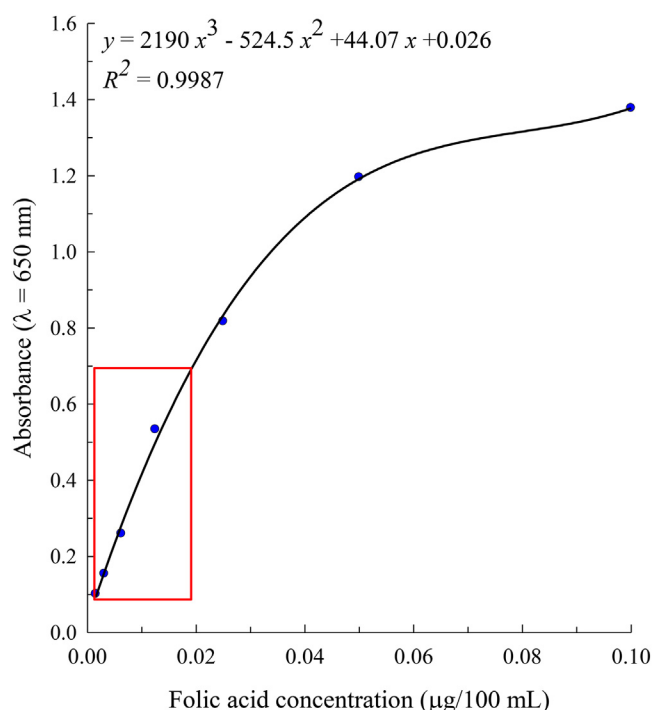


Fig. 2. An example of a standard curve generated in the microbiological assay of folate concentration versus absorbance. The inserted box depicts the linear region of this polynomial curve, which can be used for folate quantification in the fruit and vegetable samples.

the curve and taking into account appropriate dilution factors and sample masses, the folate contents in the fruits and vegetables were determined and reported as μg folic acid equivalents/100 g, f. w. Because the mass of fresh and fresh-stored corn samples changed during blanching, the folate content in these was corrected according to the moisture content before and after blanching. Each sample was analyzed in triplicate.

2.7. Data analysis

The analyses of this study were performed over the span of two years in 6 distinct time frames: (1) Summer to Fall Year 1, (2) Fall to

Winter Year 1, (3) Winter to Spring Year 1, (4) Summer to Fall Year 2, (5) Fall to Winter Year 2, and (6) Winter to Spring Year 2. Within each time period, nutrient contents were measured in triplicate for all combinations of treatments and samples. Therefore, the nutrient contents of each treatment/sample combination was assessed according to 18 replications.

For each of the 8 sample-types (i.e., each specific fruit or vegetable), one-way ANOVA was performed to determine the presence of significant difference in nutrient contents according to treatment ($\alpha=0.05$). Data transformation was applied when necessary to adequately meet the assumption of normal distribution for ANOVA, and the Weighted Least Squares method was employed in the instances in which the equal variance assumption of the ANOVA was violated. All necessary statistical diagnostic checks (e.g., residuals versus predicted value plot, Q-Q plot of residuals, and histogram plot of residuals) were performed to verify acceptability of ANOVA implementation. In the cases in which a statistically significant difference was observed among the three treatments, the Tukey's Studentized Range multiple comparisons test was performed to determine which specific pairs of treatments (i.e., fresh vs. frozen, fresh vs. fresh-stored, and fresh-stored vs. frozen) showed significant differences ($\alpha=0.05$).

All data analyses were performed using SAS software, version 9 of the SAS system for Windows (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. L-Ascorbic acid

Details for the validation of the assay employed are available in the Supplementary material. The mean L-ascorbic acid contents determined in fresh, fresh-stored, and frozen broccoli, cauliflower, corn, green beans, green peas, spinach, blueberries and strawberries as well as the corresponding fresh and frozen values from the USDA National Nutrient Database for Standard Reference (SR28), herein after referred to simply as Database (USDA ARS, 2015), are presented in Table 1.

There were no statistically significant differences for L-ascorbic acid contents in fresh, fresh-stored and frozen produce for broccoli, cauliflower, and corn. The L-ascorbic acid content in corn was the lowest of all produce analyzed. These values are in general agreement with the USDA Database, with the exception of fresh

Table 1
L-Ascorbic acid content (mg AA/100 g, f.w.) in selected fresh, fresh-stored, and frozen fruits.¹

Vegetables and Fruits	Mean \pm SD ²			USDA Fresh ³	USDA Frozen ⁴
	Fresh	Fresh-stored	Frozen		
Broccoli	50.2 \pm 8.47A	46.7 \pm 10.75A	50.6 \pm 3.32A	89.2 \pm 3.98(n = 19)	68.3 \pm 2.84(n = 39)
Cauliflower	45.1 \pm 6.07A	42.0 \pm 6.35A	40.4 \pm 7.50A	48.2 \pm 3.77(n = 28)	48.8 \pm 1.67(n = 34)
Corn	6.2 \pm 0.90A	5.7 \pm 0.90A	6.2 \pm 1.29A	6.8 \pm 0.57(n = 7)	6.4 \pm 1.55(n = 68)
Green beans	7.7 \pm 3.66B	6.7 \pm 3.60B	11.0 \pm 2.34A	12.2 \pm 0.75(n = 9)	12.9 \pm 1.48(n = 80)
Green peas	19.0 \pm 5.11A	15.4 \pm 3.42B	21.1 \pm 3.27A	40.0(n = 1)	18.0 \pm 0.66(n = 77)
Spinach	25.2 \pm 3.49A	19.6 \pm 5.51B	14.5 \pm 5.70C	28.1 \pm 4.13(n = 7)	5.5 \pm 0.69(n = 11)
Blueberries	13.1 \pm 4.23A	10.2 \pm 2.66A	9.9 \pm 5.71A	9.7 \pm 0.89(n = 4)	2.5 \pm 2.37(n = 3)
Strawberries	55.2 \pm 9.83A	48.3 \pm 12.21A	54.7 \pm 9.61A	58.8 \pm 2.47(n = 9)	41.2 \pm 9.06(n = 6)

¹ Abbreviations: AA—ascorbic acid; f.w.—fresh weight.

² Mean \pm SD values calculated based on 18 individual observations. Those followed by a different letter within a row (for each sample) reflect significant differences ($p < 0.05$) according to ANOVA and means separation with Tukey's Studentized Range via SAS software.

³ USDA National Nutrient Database for Standard Reference (SR28) sample means \pm standard error {SE} (n = number of data points) for: Broccoli, raw; Broccoli, frozen, chopped, unprepared; Cauliflower, raw; Cauliflower, frozen, unprepared; Corn, sweet, yellow, raw; Corn, sweet, yellow, frozen, kernels cut off cob, unprepared; Beans, snap, green, raw; Beans, snap, green, frozen, all styles, unprepared; Peas, green, raw; Peas, green, frozen, unprepared; Spinach, raw; Spinach, frozen, chopped or leaf, unprepared; Blueberries, raw; Blueberries, frozen, unsweetened; Strawberries, raw and Strawberries, frozen, unsweetened.

⁴ USDA National Nutrient Database for Standard Reference (SR28) sample means \pm standard error {SE} (n = number of data points) for: Broccoli, raw; Broccoli, frozen, chopped, unprepared; Cauliflower, raw; Cauliflower, frozen, unprepared; Corn, sweet, yellow, raw; Corn, sweet, yellow, frozen, kernels cut off cob, unprepared; Beans, snap, green, raw; Beans, snap, green, frozen, all styles, unprepared; Peas, green, raw; Peas, green, frozen, unprepared; Spinach, raw; Spinach, frozen, chopped or leaf, unprepared; Blueberries, raw; Blueberries, frozen, unsweetened; Strawberries, raw and Strawberries, frozen, unsweetened.

broccoli, for which our observed values were substantially lower than those of the database.

The frozen green beans possessed a mean L-ascorbic acid level significantly ($p < 0.05$) greater than both the fresh and fresh-stored samples. The Database also reports a mean content of vitamin C for fresh green beans being lower than that of its frozen counterpart, but this difference in the Database is not statistically significant and is of lower magnitude than the difference observed in our study. Makhlouf et al. (1995) found vitamin C in the raw beans had an average of 8.2 mg/100 g, which corresponds well to the magnitudes recorded in our study. In this study, fresh-stored green beans showed a 13% lower value of L-ascorbic acid than for fresh green beans, but this difference was not determined to be statistically significant. Fresh-stored green beans showed an L-ascorbic acid value 40% lower than that of frozen green beans, a difference determined to be statistically significant. Other researchers have reported marked L-ascorbic acid loss with extended storage (Favell, 1998; Howard et al., 1999; Spínola et al., 2012). Martins and Silva (2004) found that the beans' quality held well during frozen storage.

The absolute mean value of L-ascorbic acid in frozen green peas was greater than that of fresh peas, but the difference was not statistically significant. The Database indicates fresh green peas have much higher L-ascorbic acid contents than frozen green peas, but we did not find a supporting result here. It is worth noting that the Database reports only a single data point for fresh green peas for this nutrient, while our study found fresh green peas to have L-ascorbic acid contents highly comparable to the much more frequently reported contents found in frozen green peas. For fresh-stored green peas, the L-ascorbic acid content was significantly lower than both of the other treatment types (fresh and frozen). In other studies, frozen peas were found to have a good retention of L-ascorbic acid (Bouzari et al., 2015; Favell, 1998). A study from Hunter and Fletcher (2002) indicated a potential for dramatic loss in nutrients if fresh green peas were not properly stored. Our results suggest that in order to offer comparable L-ascorbic acid to the consumer as that found in frozen green peas, fresh green peas would need to be consumed within a short period of time after purchase.

In the case of spinach, all treatments were statistically different from one another, with fresh exhibiting the greatest values, and fresh-stored exhibiting greater values than frozen. There is variation between the values from the study and the USDA Database, but the trend of greater L-ascorbic in fresh spinach is consistent. It is worth noting that only the frozen sample was blanched before analysis. Considering the large surface area of spinach relative to the other fruits and vegetables examined in this study, it is feasible that the blanching step may explain the noted diminished quantities of L-ascorbic acid content. It was also observed that refrigerated storage is associated with substantially lower contents of L-ascorbic acid (fresh-stored contents being 22%

lower than that of the fresh samples). Giannakourou and Taoukis (2003) found higher levels of L-ascorbic acid in frozen spinach, and other studies have also noted poor retention of vitamin C in spinach during storage (Favell, 1998; Hunter and Fletcher, 2002). In a recent study, Phillips et al. (2016) found substantial losses of vitamin C in spinach samples that had been stored in refrigeration for durations of 1 and 7 days (9.5 mg/100 g and 31 mg/100 g, respectively). Considering the losses of L-ascorbic acid post-harvest and in refrigerated storage, freezing could still impart an advantage compared to refrigeration, although our results suggest this would likely primarily only be the case in situations where the consumer will eventually either blanch their fresh spinach or store it for extended periods following purchase.

There was no statistically significant difference in the levels of L-ascorbic acid for the two berry types. Our findings suggest a greater similarity in the L-ascorbic acid content in fresh blueberries and strawberries as compared to their frozen counterparts than is reported in the USDA Database.

3.2. *Trans*- β -carotene

Details for the validation of the assay employed are available in the Supplementary material. Table 2 summarizes the *trans*- β -carotene content of fresh, fresh-stored, and frozen produce based on the f.w. for broccoli, cauliflower, corn, green beans, green peas, spinach, blueberries and strawberries. The *trans*- β -carotene contents within the assessed sample types showed high variances, as can be seen from the relatively high standard deviation values (standard errors frequently greater than 20%). This observation may be at least partly attributable to the established characteristic of *trans*- β -carotene concentrations to be affected by factors such as storage time and storage conditions, as this could feasibly result in substantial inconsistencies of *trans*- β -carotene concentrations within available produce (Villa-Rodriguez et al., 2015).

For broccoli, there were no statistically significant differences between the fresh and fresh-stored test samples. Frozen broccoli was lower and statistically different from its fresh counterparts. Biehler et al. (2010) similarly reported that the *trans*- β -carotene content in frozen broccoli was lower than its fresh analog. Fresh broccoli stored at 4 °C for 5 days after purchase did not show a significant reduction in *trans*- β -carotene. Howard et al. (1999) reported no pattern of retention was evident. Hussein et al. (2000) found a decrease of 10% in *trans*- β -carotene content in fresh broccoli stored at 4 °C for 10 days. Different packaging may result in differences on the retention of total carotenoids during transport after processing and storage. Specifically, Barth and Zhuang (1996) found that modified atmosphere packaging prevented any loss in carotenoids of broccoli florets stored at 5 °C for 6 days, while unwrapped florets or florets wrapped in perforated film lost ~50% of their carotenoid content under the same conditions. Other

Table 2
trans- β -Carotene content ($\mu\text{g}/100\text{ g}$, f.w.) in selected fresh, fresh-stored, and frozen fruits.¹

Vegetables and Fruits	Mean \pm SD ²			USDA Fresh ³	USDA Frozen ⁴
	Fresh	Fresh-stored	Frozen		
Broccoli	2020 \pm 390A	1710 \pm 300A	940 \pm 200B	360 \pm 10 (n = 124)	675 (Imputed)
Cauliflower	24.2 \pm 11.4A	17.8 \pm 12.6A	16.5 \pm 7.7A	0 \pm 0.01 (n = 4)	7 (Imputed)
Corn	65.4 \pm 34.1AB	35.7 \pm 20.0B	77.2 \pm 34.0A	47 (Imputed)	49 (Imputed)
Green beans	501 \pm 70.5A	360 \pm 144B	359 \pm 117B	379 \pm 49 (n = 77)	292 (n = 5)
Green peas	957 \pm 343A	610 \pm 150B	1084 \pm 224A	449 \pm 109 (n = 40)	1225 (Imputed)
Spinach	11100 \pm 2200A	8900 \pm 1720B	8500 \pm 2280B	5626 \pm 770 (n = 5)	7035 (Imputed)
Blueberries	196 \pm 43.6A	136 \pm 25.7B	74 \pm 24.3C	32 \pm 3.3 (n = 16)	28 (Imputed)
Strawberries	21.2 \pm 6.5A	13.2 \pm 4.0B	20.7 \pm 10.5A	7 \pm 0.2 (n = 8)	27 (Imputed)

¹⁻⁴See footnotes of Table 1 for description of the samples reported from the USDA National Nutrient Database for Standard Reference (SR28).

studies reported better stability of carotenoids in frozen broccoli (Martin et al., 1960; Wu et al., 1992).

For cauliflower, the mean *trans*- β -carotene content in fresh products was found to be $24.2 \pm 11.4 \mu\text{g}/100 \text{ g, f.w.}$, whereas in the Database it is listed as zero based on 4 observations. Other studies have also found detectable levels of *trans*- β -carotene in fresh cauliflower (Gębczyński and Kmiecik, 2007; Kurilich et al., 1999). Our findings showed that the *trans*- β -carotene contents in fresh, fresh-stored, and frozen cauliflower were not statistically significantly different.

The *trans*- β -carotene content of frozen corn was not significantly different from that of fresh corn, but was significantly greater than that of the fresh-stored corn. A study by Scott and Eldridge (2005) reported a higher *trans*- β -carotene content in frozen golden whole kernel corn and white shoepeg compared to that of their fresh counterparts. The higher quantities of *trans*- β -carotene observed in frozen corn may be a result of water loss from the kernels, because the blanching and freezing process can dehydrate vegetables slightly. In fresh corn, the *trans*- β -carotene contents observed in our study showed a very large range (20.2 to $111 \mu\text{g}/100 \text{ g, f.w.}$). The wide range observed may possibly be attributable to the variation in cultivar and region of origin for corn-on-the-cob available in supermarkets throughout a calendar year. In this study, the fresh-stored corn on the cob showed *trans*- β -carotene contents 45% lower than that of fresh, and 54% lower than frozen. This loss of *trans*- β -carotene associated with refrigerated storage of corn-on-the-cob is the highest among all of the fresh fruits and vegetables examined in this study. Unless fresh corn is to be consumed very shortly after purchase, the results here suggest there may be a nutritional advantage, in regards to *trans*- β -carotene, of frozen corn-on-the-cob.

The fresh green beans possessed a statistically greater content of *trans*- β -carotene than both the fresh-stored and frozen produce, which themselves were not statistically different. The fresh-stored green beans showed *trans*- β -carotene levels 23% lower than that found in fresh green beans. A somewhat similar study by Howard et al. (1999) reported an average loss of 10% for *trans*- β -carotene in green beans refrigerated for 16 days.

Of all of the fruits and vegetables examined, spinach contained the highest content of *trans*- β -carotene. As with green beans, the fresh spinach had a statistically significant greater content of *trans*- β -carotene than the fresh-stored and frozen produce, which themselves were not statistically different. Pandrangi and LaBorde (2004) found *trans*- β -carotene contents in fresh spinach ranging from 5400 to $12,700 \mu\text{g}/100 \text{ g, f.w.}$, which is compatible with our findings. In our study, fresh-stored spinach corresponded to a 20% lower quantity of *trans*- β -carotene than in fresh spinach. Simonetti et al. (1991) reported a 10% decrease in *trans*- β -carotene content of spinach based on wet weight over three weeks of storage. Pandrangi and LaBorde (2004) similarly reported an 84.3% retention of carotenoids in fresh spinach stored at 4°C for 8 days.

The values of *trans*- β -carotene in frozen green peas and fresh green peas were not statistically different from one another, but the contents of both were significantly greater than that of the fresh-stored produce. Fresh-stored green peas had 36% lower *trans*- β -carotene values than the fresh green pea samples, and 44% lower than the frozen samples. This finding is compatible with a study by Simonetti et al. (1991), who also found a significant decrease of *trans*- β -carotene in peas after 3 weeks of refrigerated storage. The results of this study suggest that, when considering the time a consumer may store their fresh green peas prior to consumption, frozen green peas can present a nutritional advantage in regards to *trans*- β -carotene content.

The mean values for fresh ($196 \mu\text{g}/100 \text{ g, f.w.}$), fresh-stored ($136 \mu\text{g}/100 \text{ g, f.w.}$), and frozen blueberries ($74 \mu\text{g}/100 \text{ g, f.w.}$) were each statistically different from one another. Fresh-stored blueberries showed *trans*- β -carotene contents 31% below that of fresh blueberries, and the frozen blueberries had contents 45% below that. For strawberries, the fresh-stored sample was significantly lower than fresh and frozen, while fresh and frozen were not significantly different from one another. The *trans*- β -carotene in fresh-stored strawberries was found to be 38% below that of fresh and 36% below that of frozen. In both fruits investigated, we observed *trans*- β -carotene degradation to be quite pronounced after harvesting.

3.3. Folate

Details for the validation of the assay employed are available in the Supplementary material. Table 3 summarizes the mean folate contents of fresh, fresh-stored, and frozen produce based on the f. w. for broccoli, cauliflower, corn, green beans, green peas, spinach, blueberries and strawberries. For broccoli, there was no statistically significant difference between the fresh and fresh-stored test samples, but frozen broccoli was lower in folate and statistically different from its fresh counterparts. It should be noted that neither the fresh nor fresh-stored broccoli analyzed in this study was blanched, as samples from the composite were immediately placed in a phosphate buffer. The frozen broccoli, however, had been blanched prior to freezing and this may have contributed to the lower mean folate values observed here in frozen samples. Studies have indicated a loss of folate resulting from blanching of fresh broccoli. Specifically, DeSouza and Eitenmiller (1986) observed losses of 60 and 9% after water and steam blanching, respectively, compared with fresh broccoli.

For cauliflower, no statistically significant difference was found between folate values in fresh, fresh-stored, and frozen produce. Other studies have reported higher folate contents in fresh cauliflower than those in this work (Puupponen-Pimiä et al., 2003; Vahteristo et al., 1997). It was suggested that the difference in folate contents was due to variability in the cultivars tested. It should be pointed out, however, that the microbiological assay for

Table 3
Food/total folate content ($\mu\text{g}/100 \text{ g, f.w.}$) in selected fresh, fresh-stored, and frozen fruits.¹

Vegetables and Fruits	Mean \pm SD ²			USDA Fresh ³	USDA Frozen ⁴
	Fresh	Fresh-stored	Frozen		
Broccoli	72.6 \pm 7.68A	67.4 \pm 8.68AB	61.7 \pm 9.28B	63 \pm 12.7(n=27)	94 \pm 9.2(n=9)
Cauliflower	68.9 \pm 4.40A	65.0 \pm 5.49A	67.7 \pm 8.47A	57 \pm 5.3(n=27)	64(Imputed)
Corn	50.2 \pm 10.5A	37.2 \pm 10.1B	50.6 \pm 6.73A	42 \pm 3.4(n=7)	36 \pm 5.5(n=6)
Green beans	36.8 \pm 10.0A	30.2 \pm 6.90AB	28.5 \pm 12.0B	33 \pm 2.7(n=8)	15 \pm 2.6(n=3)
Green peas	54.2 \pm 10.3AB	47.2 \pm 4.46B	58.1 \pm 13.1A	65(n=1)	53(Imputed)
Spinach	134 \pm 20.1A	129 \pm 16.5A	141 \pm 13.8A	194 \pm 35.6(n=6)	145 \pm 8.3(n=11)
Blueberries	11.5 \pm 4.52AB	9.6 \pm 3.79B	13.8 \pm 3.93A	6 \pm 0.1(n=12)	7 \pm 1.3(n=3)
Strawberries	43.0 \pm 12.7A	38.5 \pm 4.54A	39.7 \pm 8.50A	24 \pm 5.5(n=3)	17 \pm 5.4(n=3)

^{1–4}See footnotes of Table 1 for description of the samples reported from the USDA National Nutrient Database for Standard Reference (SR28).

folate can lead to erroneous data if the protocol is not followed carefully and a quality control plan is not in place to monitor the repeatability and precision of the assay.

The values for frozen corn and fresh corn were not statistically significantly different. However, the folate contents in fresh corn and frozen corn were both significantly greater than that of the fresh-stored produce. In this study, the folate content of fresh-stored corn-on-the-cob was 26% lower than that of fresh corn-on-the-cob, and also 26% lower than that of frozen. This loss of folate associated with refrigerated storage is, by percentage, the greatest among all of the fresh fruits and vegetables examined in this study. In regards to folate, the findings suggest a nutritional advantage in either consuming frozen corn or consuming fresh corn very shortly after purchase, rather than consuming fresh corn that has been stored for 5 days.

The fresh green beans possessed a significantly greater content of folate than the frozen produce, which in turn was not statistically different from that of the fresh-stored green beans. Again it should be noted that neither the fresh nor fresh-stored green beans analyzed in this study were blanched, as samples from the composite were immediately placed in a phosphate buffer. The frozen green beans, however, were blanched prior to freezing which could contribute to the observed lower folate contents in these samples.

Of all of the fruits and vegetables examined, spinach contained the highest content of folate. No statistically significant differences were noted in the folate content of the spinach treatments. Other studies have found high variances in spinach folate content (Pandurangi and LaBorde, 2004; Rychlik, 2004). DeSouza and Eitenmiller (1986) reported a 60% loss in folic acid for blanched spinach, whereas Cooper et al. (1978) found that blanching in boiling water for 3 min resulted in a 33% loss compared with fresh spinach. This study also found that microwave blanching without water showed a relatively minor 14% loss relative to fresh spinach. Considering the losses of folate associated with blanching, it is somewhat noteworthy that the frozen spinach, which was the only blanched sample, possessed the highest mean folate content (albeit not statistically significantly so) of all spinach samples assessed. Chen et al. (1983) found a 26% decrease in folate content of spinach stored at 4 °C for 7 days, but our study did not observe comparable losses in storage. The authors of that study suggested that blanching and immediate freezing of spinach can retain more than 80% of the folacin in fresh spinach if the air is excluded from the packaging.

The values for frozen green peas and fresh green peas were not statistically different, but the folate in frozen green peas was significantly greater than that of the fresh-stored produce. Stea et al. (2006) found 47.9 µg/100 g of folate in raw green peas. Fresh-stored green peas had values of folate 13% below that of fresh green peas, and 19% below that of frozen. Again, the frozen green peas were the only assessed sample that had undergone blanching prior to their assessment, making their high folate contents noteworthy. Selman (1994) has previously noted the folate content in green peas cooked in water to be much lower than raw green peas. Our results suggest that even if there are losses of the water-soluble folate during blanching, the frozen storage of green peas still results in folate being retained at high levels.

For blueberries, the folate content in frozen blueberries was significantly greater than that of fresh-stored blueberries. Fresh blueberries had a mean value below frozen and above fresh-stored, but the difference was not statistically significant in either case. As with other produce types assessed, the results suggest a possible nutrient advantage for frozen when considering the losses of nutrients during refrigerated storage. For strawberries, there were no significant differences observed among the three treatments.

4. Conclusions

In our comparisons of the levels of L-ascorbic acid, trans-β-carotene, and folate found in fresh, fresh-stored, and frozen fruits and vegetables, we determined that the majority of comparisons yielded no significant difference. In the cases of significant differences, there was a generally consistent observation of five days of refrigerated storage having a negative association with nutrient concentration. Corresponding to this observed negative association, our study found frozen produce samples to have significantly higher nutrient contents than fresh-stored more frequently than the inverse. Overall, our findings suggest that the time a consumer stores their fresh produce prior to consumption is an important factor in determining comparative nutritional value, and one that we believe merits more consideration from investigators when making nutritional comparisons. When accounting for a storage period that mimics that employed by consumers, our findings do not support the common perception that fresh produce is nutritionally superior to frozen produce.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jfca.2017.02.002>.

References

- AOAC International, 2006a. AOAC Official Method 967.21, L-Ascorbic Acid in Vitamin Preparations and Juices 2,6-Dichloroindophenol Titrimetric Method, 45.1.14. Association of Official Analytical Chemists International, Arlington, VA.
- AOAC International, 2006b. AOAC Official Method 984.325, Moisture (Loss of Mass on Drying) in Frozen French-fried Potatoes. Association of Official Analytical Chemists International, Arlington, VA.
- AOAC, 1998. AOAC Peer-Verified Methods Program. Manual on Policies and Procedures. Association of Official Analytical Chemists, Arlington, VA.
- Agarwal, S., Reider, C., Brooks, J.R., Fulgoni III, V.L., 2015. Comparison of prevalence of inadequate nutrient intake based on body weight status of adults in the United States: an analysis of NHANES 2001–2008. *J. Am. Coll. Nutr.* 34, 126–134.
- Alvarez-Suarez, J.M., Mazzoni, L., Forbes-Hernandez, T.Y., Gasparrini, M., Sabbadini, S., Giampieri, F., 2014. The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: a review. *J. Berry Res.* 4, 1–10.
- Ares, G., De Saldamando, L., Giménez, A., Deliza, R., 2014. Food and wellbeing: towards a consumer-based approach. *Appetite* 74, 61–69.
- Barth, M.M., Zhuang, H., 1996. Packaging design affects antioxidant vitamin retention and quality of broccoli florets during postharvest storage. *Postharvest Biol. Tec.* 9, 141–150.
- Biehler, E., Mayer, F., Hoffmann, L., Krause, E., Bohn, T., 2010. Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. *J. Food Sci.* 75, C55–C61.
- Blackburn, J., Scudder, G., 2009. Supply chain strategies for perishable products: the case of fresh produce. *Prod. Oper. Manag.* 18, 129–137.

- Bouzari, A., Holstege, D., Barrett, D.M., 2015. Vitamin retention in eight fruits and vegetables: a comparison of refrigerated and frozen storage. *J. Agric. Food Chem.* 63, 957–962.
- Brandwein, M., Al-Quntar, A., Goldberg, H., Mosheyev, G., Goffer, M., Marin-Iniesta, F., López-Gómez, A., Steinberg, D., 2016. Mitigation of biofilm formation on corrugated cardboard fresh produce packaging surfaces using a novel thiazolidinedione derivative integrated in acrylic emulsion polymers. *Front. Microbiol.* 7, 1–9.
- Buzby, J.C., Farah-Wells, H., Hyman, J., 2014. The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. *USDA-ERS Econ. Inform. Bull.* 121.
- CDC (National Center for Chronic Disease Prevention and Health Promotion, Division of Nutrition, Physical Activity, and Obesity), 2013. *State Indicator Report on Fruits and Vegetables 2013*.
- Chen, T.S., Song, Y.O., Kirsch, A.J., 1983. Effects of blanching, freezing and storage on folacin content of spinach. *Nutr. Rep. Int.* 28, 317–324.
- Cooper, R.G., Chen, T.S., King, M.A., 1978. Thermal destruction of folacin in microwave and conventional heating. *J. Am. Diet. Assoc.* 73, 406–410.
- DeSouza, S.C., Eitenmiller, R.R., 1986. Effects of processing and storage on the folate content of spinach and broccoli. *J. Food Sci.* 5 (1), 626–628.
- Deliens, T., Clarys, P., De Bourdeaudhuij, I., Deforche, B., 2014. Determinants of eating behaviour in university students: a qualitative study using focus group discussions. *BMC Pub. Health* 14 (53) (12 p).
- Favell, D.J., 1998. A comparison of the vitamin C content of fresh and frozen vegetables. *Food Chem.* 62, 59–64.
- Food Marketing Institute, 2015. *US Grocery Shopper Trends*. Food Marketing Institute.
- Fulton, S.L., McKinley, M.C., Young, I.S., Cardwell, C.R., Woodside, J.V., 2016. The effect of increasing fruit and vegetable consumption on overall diet: a systematic review and meta-analysis. *Crit. Rev. Food Sci. Nutr.* 56, 802–816.
- Gębczynski, P., Kmiecik, W., 2007. Effects of traditional and modified technology, in the production of frozen cauliflower, on the contents of selected antioxidative compounds. *Food Chem.* 101, 229–235.
- Giannakourou, M.C., Taoukis, P.S., 2003. Kinetic modelling of vitamin C loss in frozen green vegetables under variable storage conditions. *Food Chem.* 83, 33–41.
- Haynes-Maslow, L., Parsons, S.E., Wheeler, S.B., Leone, L.A., 2013. A qualitative study of perceived barriers to fruit and vegetable consumption among low-income populations, North Carolina, 2011. *Prev. Chronic Dis.* 10, 10p.
- Howard, L.A., Wong, A.D., Perry, A.K., Klein, B.P., 1999. β -Carotene and ascorbic acid retention in fresh and processed vegetables. *J. Food Sci.* 64, 929–936.
- Hunter, K.J., Fletcher, J.M., 2002. The antioxidant activity and composition of fresh frozen, jarred and canned vegetables. *Innov. Food Sci. Emerg. Technol.* 3, 399–406.
- Hussein, A., Odumeru, J.A., Ayanbadejo, T., Faulkner, H., McNab, W.B., Hager, H., Szijarto, L., 2000. Effects of processing and packaging on vitamin C and β -carotene content of ready-to-use (RTU) vegetables. *Food Res. Int.* 33, 131–136.
- Kurilich, A.C., Tsau, G.J., Brown, A., Howard, L., Klein, B.P., Jeffery, E.H., Kushad, M., Wallig, M.A., Juvik, J.A., 1999. Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *J. Agric. Food Chem.* 47, 1576–1581.
- Kyureghian, G., Stratton, J., Bianchini, A., Albrecht, J., 2010. Nutritional Comparison of Frozen and Non-frozen Fruits and Vegetables: Literature Review. The Food Processing Center University of Nebraska-Lincoln. (accessed 30 January 2017) <https://pdfs.semanticscholar.org/fd90/0931812081bff85f304a557906852b9add90.pdf>.
- Liu, R.H., 2013. Health-promoting components of fruits and vegetables in the diet. *Adv. Nutr.* 4, 384S–392S.
- Makhlouf, J., Zee, J., Tremblay, N., Bélanger, A., Marie-Hélène, M., Gosselin, A., 1995. Some nutritional characteristics of beans, sweet corn and peas (raw, canned and frozen) produced in the province of Quebec. *Food Res. Int.* 28, 253–259.
- Martin, M.E., Sweeney, J.P., Gilpin, G.L., Chapman, V.J., 1960. Factors affecting the ascorbic acid and carotene content of broccoli. *J. Agric. Food Chem.* 8, 387–390.
- Martins, R.C., Silva, C.L.M., 2004. Frozen green beans (*Phaseolus vulgaris* L.) quality profile evaluation during home storage. *J. Food Eng.* 64, 481–488.
- Oliveira, M., Abadias, M., Usall, J., Torres, R., Teixidó, N., Viñas, I., 2015. Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables—a review. *Trends Food Sci. Tech.* 46, 13–26.
- Pandurangi, S., LaBorde, L.F., 2004. Retention of folate, carotenoids, and other quality characteristics in commercially packaged fresh spinach. *J. Food Sci.* 69, C702–C707.
- Patrignani, F., Siroli, L., Serrazanetti, D.I., Gardini, F., Lanciotti, R., 2015. Innovative strategies based on the use of essential oils and their components to improve safety: shelf-life and quality of minimally processed fruits and vegetables. *Trends Food Sci. Technol.* 46, 311–319.
- Phillips, K.M., Council-Troche, M., McGinty, R.C., Rasor, A.S., Tarrago-Trani, M.T., 2016. Stability of vitamin C in fruit and vegetable homogenates stored at different temperatures. *J. Food Compos. Anal.* 45, 147–162.
- Puupponen-Pimiä, R., Häkkinen, S., Aarni, M., Suortti, T., Lampi, A.-M., Eurola, M., Piironen, V., Nuutila, A.M., Oksman-Caldentey, K.-M., 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *J. Sci. Food. Agric.* 83, 1389–1402.
- Rychlik, M., 2004. Revised folate content of foods determined by stable isotope dilution assays. *J. Food Compos. Anal.* 17, 475–483.
- Sahu, R.K., Agrawal, A.K., Sinha, G., 2016. Extension of shelf life of custard apple (*Annona squamosa* L.) through post harvest treatments. *Madras Agric. J.* 103, 62–66.
- Scott, C.E., Eldridge, A.L., 2005. Comparison of carotenoid content in fresh, frozen and canned corn. *J. Food Compos. Anal.* 18, 551–559.
- Selman, J.D., 1994. Vitamin retention during blanching of vegetables. *Food Chem.* 49, 137–147.
- Simonetti, P., Porrini, M., Testolin, G., 1991. Effect of environmental factors and storage on vitamin content of *Pisum sativum* and *Spinacia oleracea*. *Ital. J. Food Sci.* 3, 187–196.
- Spínola, V., Mendes, B., Câmara, J.S., Castilho, P.C., 2012. An improved and fast UHPLC-PDA methodology for determination of L-ascorbic and dehydroascorbic acids in fruits and vegetables. Evaluation of degradation rate during storage. *Anal. Bioanal. Chem.* 403, 1049–1058.
- Stea, T.H., Johansson, M., Jägerstad, M., Frølich, W., 2006. Retention of folates in cooked, stored and reheated peas: broccoli and potatoes for use in modern large-scale service systems. *Food Chem.* 101, 1095–1107.
- Stok, F.M., de Ridder, D.T.D., de Vet, E., de Wit, J.B.F., 2014. Don't tell me what I should do, but what others do: the influence of descriptive and injunctive peer norms on fruit consumption in adolescents. *Br. J. Health Psych.* 19, 52–64.
- US Department of Agriculture, Agricultural Research Service (2015). *USDA National Nutrient Database for Standard Reference, Release 28*. Retrieved September 30, 2016 from the USDA Food Composition Databases Home Page: <https://ndb.nal.usda.gov/ndb/>.
- US Department of Health and Human Services and U.S. Department of Agriculture (2015). *2015–2020 Dietary Guidelines for Americans, 8th Edition*.
- Vahteristo, L., Lehtikoinen, K., Ollilainen, V., Varo, P., 1997. Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. *Food Chem.* 59, 589–591.
- Villa-Rodríguez, J.A., Palafox-Carlos, H., Yahia, E.M., Ayala-Zavala, J.F., Gonzalez-Aguilar, G.A., 2015. Maintaining antioxidant potential of fresh fruits and vegetables after harvest. *Crit. Rev. Food Sci. Nutr.* 55, 806–822.
- Wu, Y., Perry, A.K., Klein, B.P., 1992. Vitamin C and β -carotene in fresh and frozen green beans and broccoli in a simulated system. *J. Food Quality* 15, 87–96.
- YMC™ Separation Technology, 2007. Technical Note: Separation of Carotenoids Found in Algae. YMC™ Separation Technology.