JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 6975−6985

pubs.acs.org/JAFC

Energy Regulated Nutritive and Antioxidant Properties during the Germination and Sprouting of Broccoli Sprouts (*Brassica oleracea* var. *italica*)

Lin Chen,[†,‡] Glenna Jue Tong Tan,[†] Xinyi Pang,[†] Wenqian Yuan,[†] Shaojuan Lai,[§] and Hongshun Yang*[,†,‡]

[†]Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore

[‡]National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P. R. China

[§]Guangzhou Pulu Medical Technology Co., Ltd., Guangzhou, Guangdong 510800, P. R. China

ABSTRACT: The role of energy status in germination and sprouting of broccoli seeds was investigated by exogenous ATP and DNP treatments. With the synthesis of adenylates from 38.82 to 142.69 mg·100 g^{-1} DW, the nutritive components (soluble sugar, proteins, pigments, and phenolics) and AAs were increased during germination and early sprouting (day 5). Elements of the *BoSnRK2* pathway were down-regulated by more than 2 fold under the energy charge feedback inhibition. At the end of sprouting (day 7), energy depletion resulted in slowdown or reduced nutritional accumulation and antioxidant capacities. Exogenous ATP depressed the *BoSnRK2* pathway by maintaining the energy status at high levels and further promoted the nutrition and antioxidant levels. It also prevented the energy depletion at day 7. On the contrary, DNP reduced the ATP contents (16.10−26.86%) and activated the *BoSnRK2* pathway. It also notably suppressed the energy-consuming activities including germination, sprouts growth, and secondary metabolic synthesis.

KEYWORDS: *antioxidant capacity, ATP, metabolism, transcriptional regulation, vegetable*

INTRODUCTION

Consumption of fresh produce has increased dramatically due to the health demand of consumers.[1] Broccoli sprouts have caught the public attention recently due to their high nutrition and convenience. Sufficient studies show that this micro green is rich in phenolic acids, flavonoids, trace elements, and vitamins.[2,3] In addition, the epidemiological evidence concludes that the risks of chronic diseases and certain cancers are negatively correlated with the consumption of broccoli sprouts because of the high content of bioactive compounds.[4] Unlike the fruits and vegetables which are seasonal and lose values during postharvest transportation and processing, the sprouts can be homemade and consumed freshly at any time though the year. All these factors make broccoli sprouts a good alternative for plant food.

Germination and sprouting of edible seeds result in complicated physiological changes. The de novo production of various bioactive compounds helps the plants against different environment stresses.[5,6] Previous studies show enhanced nutrition values and decreased contents of antinutrients in sprouts compared with ungerminated seeds. Antioxidant activities (AAs) of the germinated legumes lupin and mung bean are improved due to the increased total phenolics and flavonoid contents.[7−9] On the contrary, nonnutritional factors such as protease inhibitor and lectins are decreased during the germination of legumes. Also, flaxseeds germinated for 8 days represent the increased contents of amino acid and ascorbic acid and decreased contents of trypsin inhibitor, linustatin, and oil.[10] In addition, nutritive pigments including carotenoid and chlorophyll are synthesized during the germination period of broccoli sprouts.[2]

Energy metabolism is an important factor which regulates the vital activities in plants. Energy production and accumulation by aerobic respiration is an early event of germination, and a large amount of energy is required to start the life cycle.[11] The stored materials including carbohydrates, proteins, and lipids in seeds are utilized as nutrients and energy sources due to the heterotrophic nature at the germination stage.[12] During the early development or sprouting process, energy balance also plays a key role to improve the photosynthetic productivity.[13] However, due to the complicated energy production, transfer, and control processes, little information about energy metabolism and its role in quality formation of vegetable sprouts is available currently. Some energy regulatory elements involved in adenosine triphosphate (ATP) synthesis (ATP synthase β subunit, AtpB), degradation (alternative oxidase, AOX; mitochondrial inner membrane uncoupling proteins, UCP), transportation [adenosine diphosphate (ADP)/ATP carrier, AAC], and regulation (sucrose nonfermenting-1-related kinase, SnRK) have attracted considerable attention recently.[14] To understand the roles of these proteins in energy metabolism is crucial to clarify the key factor of energy regulation during germination and sprouting and also

Received: January 28, 2018
Revised: May 3, 2018
Accepted: June 11, 2018
Published: June 11, 2018

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

may help to gain new insights to control the development process and enhance the quality of vegetable sprouts to meet the increasing demand of ingredients and extractable nutraceutical yields in fresh produce by the public and food and drug industries.

In postharvest field, sufficient studies indicate that energy deficit or decreased energy generation efficiency result in the senescence and quality disorders of fruits and vegetables.[14,15] Low levels of ATP and energy charge (EC) also lead to the accumulation of reactive oxygen species and membrane permeability of plant cells.[16] Application of exogenous ATP could be an effective strategy to increase the intracellular ATP content and further enhance the EC. The phenolic compounds and antioxidant capacities in postharvest litchi and longan are also maintained by exogenous ATP treatment.[17,18] The browning events and pathogen infection of postharvest crops could also be effectively prevented by exogenous ATP treatment. Therefore, regulation of energy metabolism may be a potential strategy in postharvest preservation of fruits and vegetables.[19,20] However, the effect of energy supply strategy on quality control at the preharvest stage is still unknown.

The objectives of this work were to investigate the energy status and its effect on antioxidant properties and phenolics of broccoli sprouts during germination and sprouting processes by ATP and DNP (2,4-dinitrophenol, a respiratory uncoupling agent which inhibits the production of ATP) treatments. Germination and sprouting states and quality-related secondary metabolism were monitored at the physiological level. Antioxidant capacities under different treatments were comprehensively assayed by three methods. Lastly, the energy metabolism was analyzed at physiological and transcriptional levels.

MATERIALS AND METHODS

Plant Material, Treatment, and Cultivation. The broccoli seeds (*Brassica oleracea* var. *italica*) were purchased from a local seed company in Singapore. The treatment and cultivation of broccoli seeds were conducted according to the method of Baenas et al.[21] with some modifications. Broccoli seeds were first immersed in 3% (v/v) sodium hypochlorite solution for 5 min and then washed by deionized (DI) water for 3 times. They were soaked in DI water overnight at 25 °C. After that, the seeds were weighed and separated into three groups and each group contained about 4 g of seeds. In the preliminary experiment, broccoli seeds were treated by ATP or DNP at different concentrations (0.1, 0.5, 1, 2, and 4 mM) for 20 min to screen the proper conditions. After germination and sprouting for 7 days, treatment with ATP (1 mM) on broccoli seeds showed significant promotion effect on germination and sprouting; thus, this concentration was chosen as the working concentration. As a comparison, DNP treatment at 2 mM which delayed the growth development was selected as the working concentration. The seeds in each group were immersed in 200 mL of ATP (1 mM), DNP (2 mM), and DI water (control group), respectively, for 20 min based on the results of the preliminary experiment. After washing by DI water for 3 times, broccoli seeds in each group were evenly sowed on a plastic seedling-raising plate (30 cm × 22 cm) and germinated at 25 °C under darkness for 3 days. The germination rate (GR) and germination index (GI) were recorded by following formulas: GR = $N_3/N_0 \times 100\%$, where N_3 represents the number of germinated seed at day 3 and N_0 is the total number of experimental seeds; GI = $\sum(W_t/T_t)$, where W_t represents the accumulated number of germinated seeds at day t and T_t represents the time corresponding to W_t.[22] The GR and GI were measured with 90 seeds which were equally divided into three groups (each group contained 30 seeds).

The germinated seeds were further sprouted for another 4 days at 25 °C under 16/8 h light/dark photoperiod. At the first and third days during sprouting, 20 mL solutions of each treatment were evenly sprayed on the seedling-raising plates supplementarily. After sprouting, the broccoli sprouts were carefully harvested and the shoot length and fresh weight of the sprouts were recorded. The harvested sprouts were then dried in an oven at 110 °C to constant weights, and the dry weights were measured.[23] At least 10 replicates from each treated group were tested. During the 7 days of germination and sprouting, ungerminated seeds at day 1, germinated seeds at day 3, and sprouts at days 5 and 7 were gently harvested from the plastic seedling-raising plates. The collected samples were immediately frozen by liquid nitrogen and ground into powder in liquid nitrogen for RNA extraction as RNA degradation caused by thawing should be avoided during the sample preparation process. For the other biochemical analysis, the powder samples were freeze-dried before the experiments.

Quality Formation Assays. The nutrient qualities including soluble sugar, protein, chlorophyll, carotenoid, anthocyanin, and total phenolics were determined. The soluble sugar content was tested by the phenol−sulfuric acid method.[19] Briefly, 100 mg of the freeze-dried sample was homogenized in 5 mL of DI water, and the mixtures were extracted in boiling water for 30 min. The cooled extracts were then centrifuged, and the supernatants were collected. A total of ∼2 mL of the diluted extract was mixed with 1 mL of 9% (m/v) phenol solution and 5 mL of concentrated sulfuric acid and kept for 30 min at room temperature and then measured at 485 nm. The soluble sugar content was calculated by a glucose standard curve (30−300 μg L^{-1}, R^2 > 0.99). The method of Altisent et al.[24] was applied to measure soluble protein content, and bovine serum albumin was used as a standard (20−100 μg mL^{-1}, R^2 > 0.99).

Chlorophyll and carotenoid contents were determined by the method of Złotek et al.[25] with some modifications. A total of 100 mg of the broccoli sample was homogenized and extracted in 5 mL of 80% (v/v) acetone solution for 2 h in darkness. The sample was then centrifuged at 12 000g for 10 min at 4 °C. The absorbances of the collected supernatants were recorded at 470, 645, and 663 nm. Concentrations of chlorophyll and carotenoid were calculated by the following equations: chlorophyll = chlorophyll a + chlorophyll b = $[(12.72A_{663} - 2.59A_{645}) + (22.88A_{645} - 4.67A_{663})]$; carotenoid = $(1000A_{470} - 3.27 \times$ chlorophyll a $- 104 \times$ chlorophyll b$)/229$. The results were expressed as mg 100 g^{-1} dry weight (DW).

The method of Chen et al.[15] was employed to measure the anthocyanin and total phenolics contents (TPCs) in broccoli sprouts. The freeze-dried sample (200 mg) was homogenized in 5 mL of methanol containing 1% (v/v) HCl and then extracted for 20 min by shaking at 200 rpm. The extract was centrifuged and filtrated through a 0.45 μm filter. The absorbances at 280, 530, and 600 nm of the filtrate were recorded. Content of anthocyanin was calculated as cyanidin-3-glucoside equivalent using a molecular weight of 449.2 g mol^{-1} and molar absorptivity of 26 900 L mol^{-1} cm^{-1}. The TPC was measured by using standard curve of gallic acid (20−200 μg mL^{-1}, R^2 > 0.99) at 280 nm and expressed as mg g^{-1} DW.

Antioxidant Capacities. AAs were assayed by DPPH, ABTS, and FRAP methods. For DPPH free radical-scavenging activity assay, 3.9 mL of the DPPH radical (0.1 mM) was mixed with ∼0.1 mL of the diluted methanol extract which was extracted similarly as total phenolics assay and kept for 30 min under dark condition. The absorbance of the sample was tested at 515 nm.[26]

The ABTS cation radical-scavenging activity was tested by the method of de Souza et al.[26] with minor modifications. A total of 5 mL of the ABTS solution was mixed with 90 μL of a potassium persulfate solution (140 mM) overnight under dark conditions for ABTS cation radical generation. The mixture was then approximately diluted to get a working solution with absorbance of 0.7 ± 0.05 units at 734 nm. A total of 30 μL of diluted methanol extract was reacted with 3 mL of ABTS working solution for 20 min in the dark. A decrease in the absorbance at 734 nm was recorded.

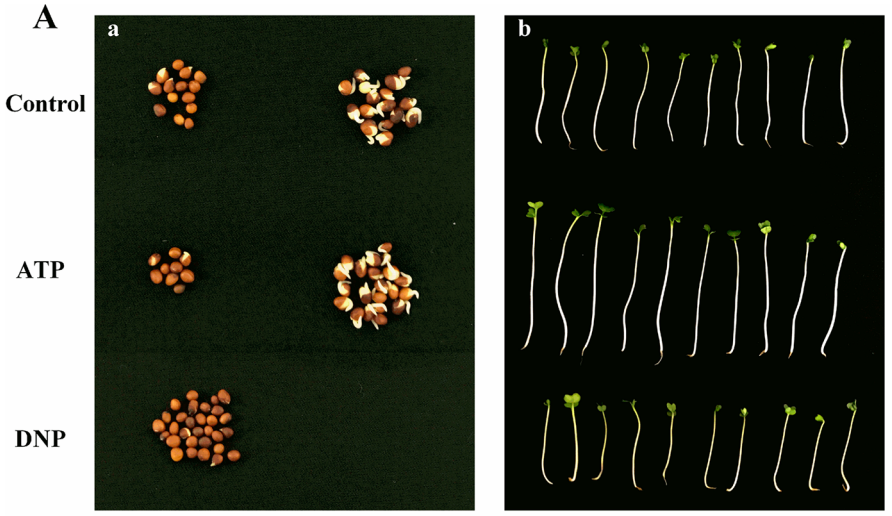
The ferric ion reducing antioxidant power (FRAP) of the methanol extract was determined according to the method of Liu et al.[27] with modifications. Briefly, acetate buffer (pH 3.6), TPTZ [2,4,6-tri(2-pyridyl)-s-triazine] solution (10 mM TPTZ in 40 mM HCl), and

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

Table 1. Primers Used for qRT-PCR of Energy-Related Genes

gene	sense primer (5′ → 3′) (forward)	antisense primer (5′ → 3′) (reverse)
BoAtpB	AACATCAACAGTTTCCAGGG	CTCGGACAAAAGCATCGGT
BoSnRK2	CCAGCGTTACAGATGCG	GACCGATAGAGTTACAAAGGA
BoACC1	GTCAGCAGGGAGAAGAGG	GAAAGGCAGTTCAATGGT
BoAOX1	CTTCTCCGTCTGGTATG	TCCGTCGTAGTCGTTT
BoUCP1	AAGACTGAGGGGATTATGG	CGAAAGCTCAGGTAACATAG

A



B

Treatments	Germination rate (%)	Germination index	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
Control	96.67 ± 3.33 a	41.17 ± 1.51 b	4.69 ± 0.40 b	24.17 ± 3.12 b	2.30 ± 0.10 b
ATP	93.33 ± 3.33 a	44.67 ± 0.50 a	5.96 ± 0.46 a	37.40 ± 4.08 a	2.70 ± 0.10 a
DNP	77.78 ± 9.62 b	20.33 ± 1.38 c	3.80 ± 0.30 c	17.71 ± 2.21 c	2.20 ± 0.00 b

Figure 1. (A) Effects of ATP and DNP on germination and sprouting of broccoli sprouts. Note: (a) Seed germination of each group at day 2; (b) harvest sprouts of each group at day 7. (B) Effect of ATP and DNP on the germination rate and index of seeds and shoot length and fresh and dry weights of sprouts. Note: different letters in B indicate significance differences for mean ($P \leq 0.05$).

ferric chloride (20 mM) solution were freshly mixed in a proportion of 10:1:1 as the working solution. Then, 100 μL of methanol extract was reacted with 900 μL of the working solution for 20 min at 37 °C, and the mixture was further tested at 593 nm to monitor the developed blue color of the reduced ferrous form.

Methanolic solutions of Trolox with different concentrations were applied for calibration of ABTS, DPPH, and FRAP tests. The results were expressed as mg Trolox g^{-1} DW.

Analysis of Free Phenolic Acids and Flavonoids. Free phenolic acids were determined by high-performance liquid chromatography (HPLC; Alliance 2695, Waters, MA, U.S.A.) using UV detection. The method of Pająk et al.[28] was applied with modifications. The organic solvent in total phenolics extract mentioned above was moved by a rotary vacuum evaporator (Rotavapor R-300, BÜCHI, Flawil, Switzerland). The obtained residue was redissolved in 200 μL of methanol and kept under −20 °C and analyzed in 24 h.

The samples (20 μL) were separated on a 5 μm C18(2) column (150 × 4.6 mm, Luna, CA, U.S.A.) at the temperature of 30 °C. Solvents A [2.5% (m/v) acetic acid solution] and B (acetonitrile) were applied for the separation with gradient elution at a flow rate of 1 mL min^{-1}. The following separable procedure was conducted: in the first 10 min, mobile phase B increased to 8% linearly, followed by increasing to 15, 20, 30, and 40% at 20, 30, 40, and 50 min, respectively. After holding for another 10 min, the column was eluted and equilibrated for 10 min before the next injection.

Phenolic acids including chlorogenic, caffeic, P-coumaric, ferulic, and sinapic acids were monitored at 320 nm, and gallic acid was determined at 280 nm. Flavonoids such as quercetin and kaempferol were detected at 360 nm. The qualitative analysis of individual phenolic acids and flavonoids was calculated by calibration curves at concentrations in the range of 0−200 μg mL^{-1}.

HPLC Analysis of ATP, ADP, and AMP. ATP, ADP, and adenosine monophosphate (AMP) were extracted by perchloric acid solution.[29] The freeze-dried powder (100 mg) was ground and extracted with 5 mL of 0.6 M perchloric acid, and the homogenate was centrifuged at 12 000*g* under 4 °C for 10 min. Then 3 mL of supernatant was then neutralized to pH 6.5−6.8 and diluted to 4 mL. After passing through a 0.45 μm filter, the solution was stored under −20 °C.

A Waters 2695 HPLC system with a Luna C18 column (150 × 4.6 mm) and a UV detector at 254 nm was used to measure the adenylate contents. Solvents A (0.05 M phosphate buffer, pH 7.0) and B (acetonitrile) were applied for the gradient elution by the following program: in the first 6 min, solvent B increased from 0 to 25% linearly and hold for another 3 min. The final program (100% A and 0% B) was taken for 1 min before the next injection. The injected sample volume was 20 μL, and the flow rate was 1.2 mL min^{-1}. The ATP, ADP, and AMP contents were calculated by the external standard curve (0.5−10 μg mL^{-1}, $R^2 > 0.99$) and expressed as DW basis. Energy charge was determined as [(ATP) + 0.5(ADP)]/[(ATP) + (ADP) + (AMP)].

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

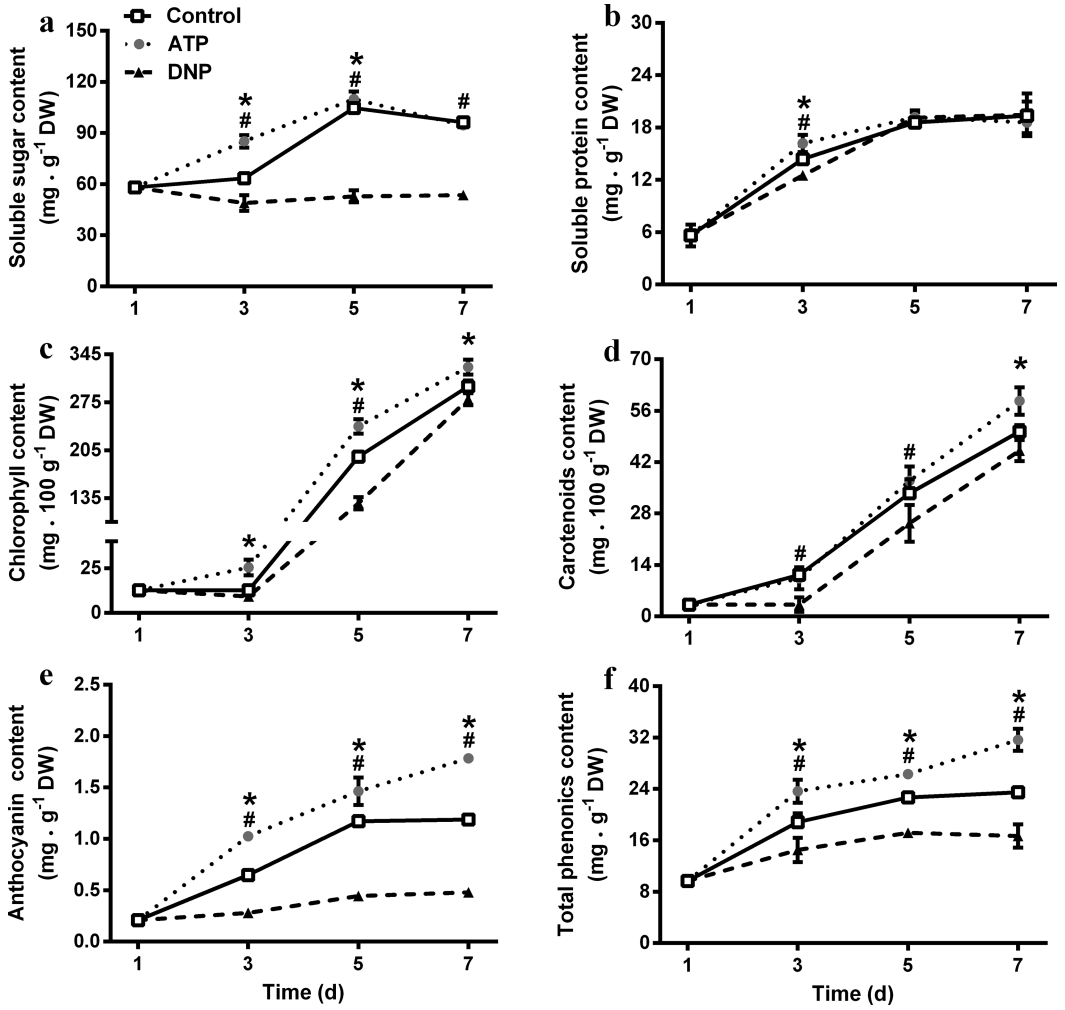


Figure 2. Effects of ATP and DNP on the contents of soluble sugar, protein, pigments (chlorophyll, carotenoid, and anthocyanin) and total phenolics. Note: the mark * (ATP treated group) or # (DNP treated group) indicate significant ($P \leq 0.05$) differences as compared to the control group at each production time.

Determination of Energy-Related Gene Transcript Levels. The expression patterns of energy-related genes were measured by quantitative RT-PCR. Total RNA was isolated from frozen broccoli seeds and sprout samples without freeze-drying by using a Total RNA Mini-Prep Kit (Bio Basic, Ontario, Canada). The concentration and quality of RNA were tested by a spectrophotometer (BioDrop, Biochrom, Cambridge, U.K.), and the integrity was checked by 1% (w/v) agarose gel electrophoresis. About 2 μg of RNA was used to synthesize cDNA by a First Strand cDNA Synthesis Kit (Sangon Biotech, Shanghai, China). The broccoli *Actin* gene (GenBank accession: AF044573) was used as internal control. Energy-related genes in *Arabidopsis thaliana* and litchi were selected and further compared with the whole genome of *Brassica oleracea* in NCBI to obtain the homologous genes in broccoli.[14] Primer pairs of energy-related genes were designed by Primer 5 software based on the whole genome information on *Brassica oleracea* in GenBank (Table 1). Furthermore, the specificities of the designed primers were verified by Primer Blast in NCBI. The reaction system and condition were conducted by the previous report, and the relative expression levels of tested genes were calculated by $2^{-\Delta\Delta CT}$ method.[30] Three biological replicates were performed for each reaction.

Statistical Analysis. Data were statistically analyzed by analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) method to assess the effects of energy regulation on broccoli germination and sprouting. In addition, differences with $P \leq 0.05$ were considered significant.

RESULTS

Germination and Sprouting Analysis. The effects of ATP and DNP treatments on the germination and sprouting of broccoli sprouts are shown in Figure 1. The results indicated that, at the germination stage, exogenous ATP treatment had no effect on the GR but significantly improved the GI from 41.17 to 44.67 (Figure 1B). DNP treatment significantly ($P \leq 0.05$) decreased the GR and GI by 18.89 and 50.62%, respectively, compared with that for the control group. Results of sprouting parameters revealed that the shoot length and fresh and dry weights in the ATP treated group were 27.1, 54.7, and 17.4%, respectively, higher than those for the control group ($P \leq 0.05$). On the contrary, the shoot length and fresh weight were significantly decreased by 19.0 and 26.7%, respectively, by DNP treatment.

Quality Formation Analysis. Changes of different nutritive factors during germination and sprouting are presented in Figure 2. The results demonstrated that contents of nutritive factors including chlorophyll and carotenoids were elevated to different degrees in the control group during the production process. Furthermore, the contents of soluble sugar, protein, anthocyanin, and total phenolics were increased from day 1 to day 5 but maintained at similar levels or even reduced at day 7 when compared with contents at day 5.

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

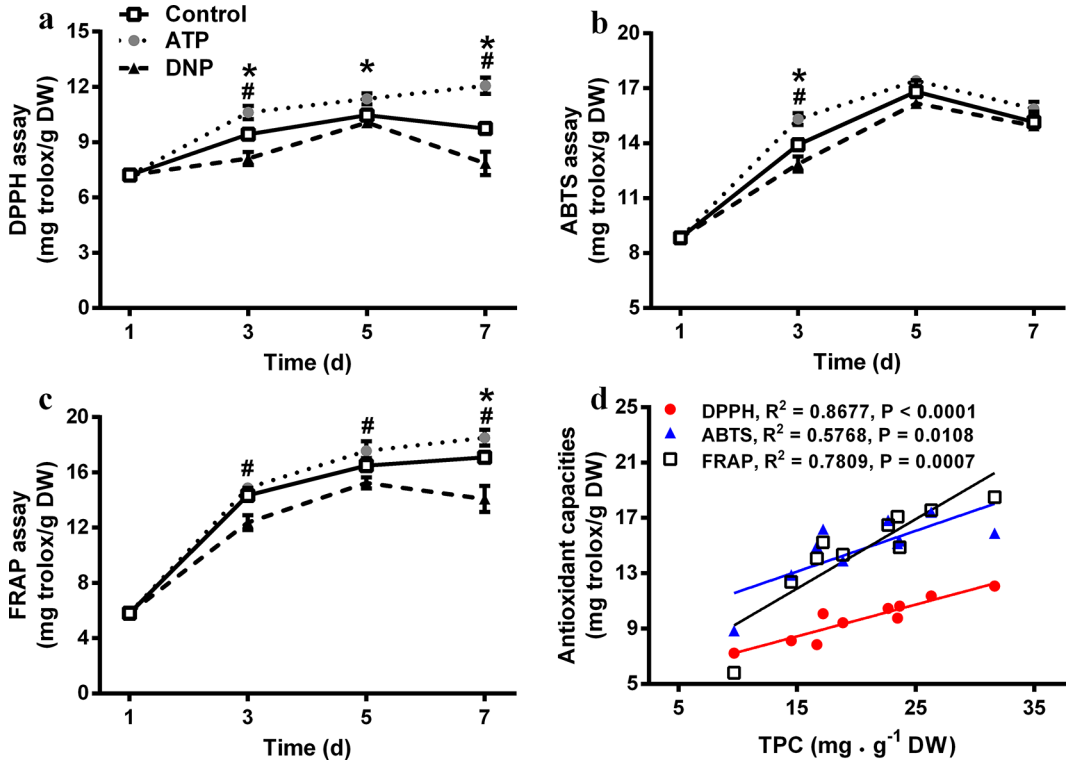


Figure 3. Effect of ATP and DNP on the antioxidant capacities assayed by DPPH, ABTS and FRAP methods and their linear correlations with total phenolics content (TPC). Note: the mark * (ATP treated group) or #(DNP treated group) indicate significant ($P \leq 0.05$) differences as compared to the control group at each production time.

For soluble sugar, exogenous ATP effectively increased the contents at the end of germination (day 3) and sprouting (day 5) by 34.21 and 4.96%, respectively, compared with the control group at the same day. However, similar levels of soluble sugar contents between the ATP treated group and the control group were observed at day 7. DNP treatment constantly maintained the sugar contents at relative low levels, which were 22.84−49.54% lower than the control group at the same test day. In addition, ATP treatment significantly ($P \leq 0.05$) elevated the soluble protein content from 14.38 to 16.18 mg g^{-1} DW at day 3 and DNP remarkably ($P \leq 0.05$) reduced it to 12.52 mg g^{-1} DW. Nevertheless, no significant differences in soluble protein contents among the three treated groups were observed at days 5 and 7.

In terms of pigments in broccoli sprouts, ATP treatment resulted in the continuously improved amounts of chlorophyll and anthocyanin, making them 9.54−100.65% and 24.79−58.25% higher, respectively, than the for those of the control during 7 days' production. For carotenoids content, ATP treatment did not alter it significantly at days 3 and 5 but significantly improved it by 16.56% at day 7 ($P \leq 0.05$). Conversely, DNP treatment reduced the contents of the three kinds of pigments at some time points. Chlorophyll content in the DNP treated group at days 3 and 7 showed no significant difference compared with that for the control group, but it was remarkably reduced by 43.52% compared with that for the control at day 5. Also, DNP treatment significantly ($P \leq 0.05$) decreased the carotenoids content by 71.87% and 24.39% at days 3 and 5, respectively. However, at day 7, no significant difference of carotenoids content between DNP and the control groups was observed. DNP treatment constantly reduced the anthocyanin contents during the production process, which were 56.70−62.11% lower than that for the control group at the same day.

The results of TPCs showed that, during the 7 days of development, ATP treatment significantly ($P \leq 0.05$) improved the contents by 16.06−34.80% in comparison with that for the control group at the same day while DNP treatment reduced these antioxidants by 23.11−28.99%.

Analysis of AAs. Three methods were applied to test the antioxidant capacities of methanol extracts of broccoli seeds and sprouts. Improved antioxidant capacities during germination and early stage of sprouting were generally recorded in three assays (Figure 3). The DPPH scavenging results demonstrated that, in the control group, an increase from 7.22 to 10.46 mg trolox g^{-1} DW in 5 days of growth was observed, followed by a slight decrease to 9.75 mg trolox g^{-1} DW at day 7. Compared with the control group at the same day, ATP treatment significantly ($P \leq 0.05$) enhanced the AAs by 8.50−23.77% during the germination and sprouting. However, DNP treatment decreased the antioxidant capacities by 13.90 and 19.45% at days 3 and 7, respectively. No significant difference of AA between the control and DNP treated groups at day 5 was observed. Similarly, AAs tested by ABTS revealed a rise from 8.84 to 16.82 mg trolox g^{-1} DW during 5 days' production and a reduction to 15.15 mg trolox g^{-1} DW at the end of sprouting in the control group. While exogenous ATP treatment improved the AAs significantly ($P \leq 0.05$) by 10.11% at day 3, DNP remarkably ($P \leq 0.05$) reduced the antioxidant capacities by 7.47%. However, there were no significant differences of AAs among the control, ATP, and DNP treated groups at days 5 and 7.

FRAP results showed a continuous increase in AAs from 5.81 to 17.09 mg trolox g^{-1} DW in the control group.

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

Table 2. Free Phenolic Acids and Flavonoids Content of Broccoli Seeds and Sprouts Extracts under Different Treatments[a]

treatments		phenolic acids (mg 100 g^{-1} DW)							flavonoids (mg 100 g^{-1} DW)	
		chlorogenic	caffeic	P-coumaric	gallic	sinapic	ferulic	total	kaempferol	quercetin
day 1	seed	146.95^{a}	1.75^{g}	0.78^{e}	0.18^{f}	0.29^{d}	0.01^{d}	149.96^{a}	ND	ND
day 3	control	71.81^{d}	22.54^{de}	0.59^{g}	1.13^{cd}	0.17^{f}	0.05^{ab}	96.29^{c}	ND	ND
	ATP	77.90^{c}	27.38^{bc}	0.85^{d}	1.40^{b}	0.23^{e}	0.05^{bc}	107.81^{c}	ND	ND
	DNP	106.10^{b}	15.90^{f}	0.65^{f}	0.79^{e}	0.14^{f}	0.04^{bc}	123.62^{b}	ND	ND
day 5	control	13.04^{f}	29.79^{b}	0.93^{c}	0.85^{e}	0.35^{c}	0.01^{d}	44.97^{e}	ND	ND
	ATP	17.28^{f}	38.93^{a}	1.24^{a}	1.34^{bc}	0.48^{b}	0.05^{ab}	59.32^{d}	ND	ND
	DNP	37.51^{e}	26.15^{c}	0.78^{de}	1.03^{de}	0.64^{a}	0.04^{c}	66.15^{d}	ND	ND
day 7	control	5.79^{g}	21.63^{e}	0.79^{de}	1.03^{de}	0.24^{de}	0.05^{ab}	29.53^{g}	0.11^{b}	0.15^{ab}
	ATP	4.68^{g}	25.43^{cd}	1.02^{b}	2.07^{a}	0.44^{b}	0.06^{a}	33.70^{f}	0.23^{a}	0.19^{a}
	DNP	4.45^{g}	14.44^{f}	0.73^{e}	2.25^{a}	0.62^{a}	0.05^{bc}	22.54^{h}	0.11^{b}	0.11^{b}

[a]With each column, values with the same superscripts do not differ significantly at $P \leq 0.05$.

Exogenous ATP significantly ($P \leq 0.05$) raised the AAs by 8.32% at day 7 compared to that for the control group, but the ATP did not cause much difference between the two groups at days 3 and 5. Inhibition of ATP synthesis by DNP treatment continuously lowered the AAs by 7.59−17.64% from day 3 to 7 compared with those for the control group at each test day. The result of linear correlations between TPC and AA evaluated by different assays is shown in Figure 3d. Statistically significant correlations ($P \leq 0.05$) were recorded in DPPH, ABTS, and FRAP tested groups (R^2 = 0.8677, 0.5768, and 0.7809, respectively).

Free Phenolic Acids and Flavonoids Profiles. The profiles of free phenolic acids and flavonoids tested by HPLC are shown in Table 2. It was found that the chlorogenic acid constituted the majority amount of phenolic acids in ungerminated broccoli seeds. During the germination and sprouting processes, the chlorogenic acid contents were sharply decreased from 146.95 to 5.79 mg 100 g^{-1} DW. ATP treatment had almost no effect on chlorogenic acid metabolism during the sprouting process. However, DNP treatment significantly slowed the decreased contents of chlorogenic acid which were 47.75 and 187.65% higher than those in control group at days 3 and 5, respectively. Caffeic acid content in broccoli was significantly increased from 1.75 to 29.79 mg 100 g^{-1} DW from day 1 to 5 but decreased to 21.63 mg · 100 g^{-1} DW at day 7. The contents of caffeic acid were 17.57−30.68% higher in the ATP treated group than those in the control at the same day. It decreased by 12.22−33.24% under DNP treatment. Also, exogenous ATP significantly ($P \leq 0.05$) enhanced the P-coumaric acid by 29.11−44.07% during the germination and sprouting of broccoli in comparison with those for the control group at the same day, while DNP reduced the content of P-coumaric acid from 0.93 to 0.78 mg 100 g^{-1} DW at day 5.

Gallic acid was synthesized more than six times during germination and the content level was maintained at around 1 mg 100 g^{-1} DW during sprouting. ATP treatment constantly induced higher gallic acid contents which were 23.89−100.97% higher than that for the control group at the same test day. DNP treatment significantly ($P \leq 0.05$) decreased the gallic acid content by 30.09% at the end of germination (day 3) and increased the content by 118.45% at day 7. Sinapic and ferulic acids in broccoli were maintained at relative low levels during germination and sprouting. Total phenolic acids (TPAs) exhibited a reduce trend ranging from 149.96 to 29.53 mg 100 g^{-1} DW in the control group, and at germination (day 3) and early sprouting (day 5) stages, DNP delayed decreases of TPAs by 28.38% and 47.10%, respectively, compared with those for the control group. In addition, exogenous ATP contributed to higher TPAs contents by 31.91 and 14.12% at the early stage (day 5) and the end (day 7) of sprouting process. Flavonoids including kaempferol and quercetin were detected only at day 7 and exogenous ATP induced kaempferol level from 0.11 to 0.23 mg 100 g^{-1} DW.

Energy Status Assays. The changes of energy status during germination and sprouting of broccoli are shown in Figure 4. Contents of ATP and ADP presented similar trends: a notable increase during 3 days' germination, followed by a slight rise at day 5, and a significant decline at the end of sprouting. After germination (day 3), exogenous ATP significantly elevated ATP (102.83 mg 100 g^{-1} DW) and ADP (25.12 mg 100 g^{-1} DW) contents to 118.87 and 29.48 mg 100 g^{-1} DW, respectively. However, the massive synthesis of ATP and ADP was significantly ($P \leq 0.05$) weakened by 16.10 and 41.87%, respectively, compared with that of the control after DNP treatment at day 3. Furthermore, after production of 7 days, the contents of ATP (75.61 mg 100 g^{-1} DW) and ADP (24.14 mg 100 g^{-1} DW) were remarkably ($P \leq 0.05$) increased by 82.92 and 54.27%, respectively, under ATP treatment and decreased to 55.30 and 17.92 mg 100 g^{-1} DW, respectively, by DNP (Figure 4a,b). A continuous elevation in AMP content was observed, and higher levels of AMP were induced by ATP (17.27−31.66%) and DNP (58.01−117.42%) treatments compared with those for the control group at the same day (Figure 4c). Total adenylate during sprouts production exhibited high similarity with changing tendencies of ATP and ADP. Also, in comparison with the control group at the same test day, ATP treatment increased it by 15.65−70.47%, while DNP treatment lowered the total adenylate content by 16.06−21.46% (Figure 4d). The energy charge was maintained at around 0.88 during the germination stage from days 1 to 3. It constantly decreased to 0.77, 0.81, and 0.67 at day 7 in the control, ATP, and DNP treated groups, respectively (Figure 4e).

Transcriptional Levels of Energy-Related Genes. Figure 5 shows the transcript abundances of energy metabolism genes including *BoAtpB*, *BoSnRK2*, *BoAAC1*, *BoAOX1*, and *BoUCP1*. Four of these genes (except *BoUCP1*) were down-regulated more than 2 fold during the germination or sprouting processes in the control group. Slight rises of *BoSnRK2* and *BoAOX1* expressions were observed at the end of sprouting. *BoAtpB* expressions in the ATP treated group were significantly reduced by 17.16% at day 3 compared with that for the control at the same day. Similar levels of

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

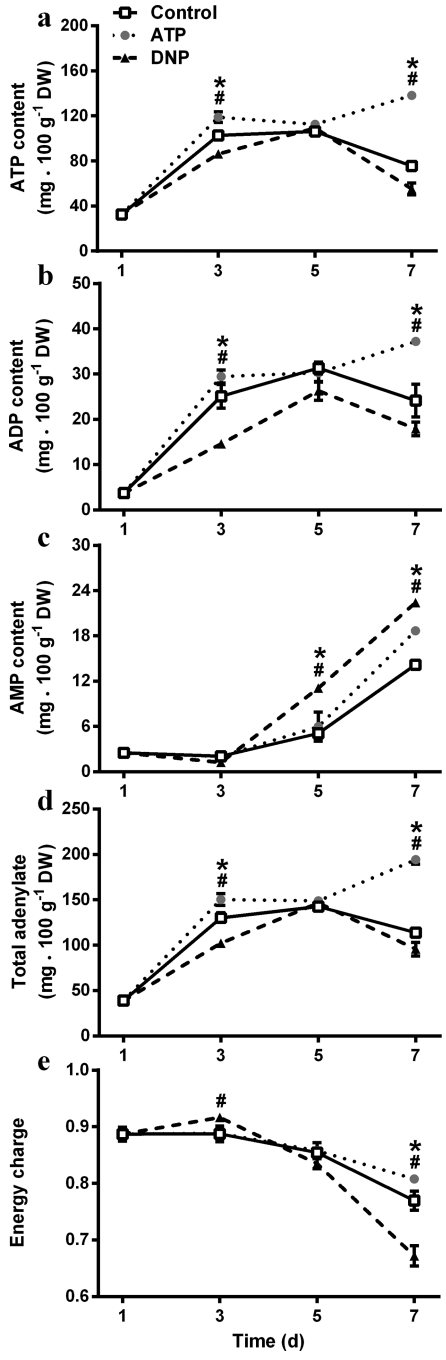


Figure 4. Effect of ATP and DNP on the ATP, ADP, AMP, and total adenylate contents and energy charge. Note: the mark * (ATP treated group) or # (DNP treated group) indicate significant ($P \leq 0.05$) differences as compared to the control group at each production time.

BoAtpB in ATP treated and control groups were observed at days 5 and 7. In addition, exogenous ATP notably ($P \leq 0.05$) depressed the *BoSnRK2* expression level by 38.08% at day 7. During the sprouting (days 5 and 7), decreases in expression levels of *BoAOX1* (23.96−37.20%) by ATP treatment were also observed. For *BoACC1* expression, the ATP treated group and the control group showed similar levels at days 5 and 7. In contrast, DNP treatment significantly induced higher expression levels of the down-regulated genes. *BoAtpB* in the DNP treated group was around 2 and 3.4 times higher compared with that for the control group at days 3 and 5, respectively. Moreover, the transcriptional levels of *BoSnRK2*, *BoACC1*, and *BoAOX1* were 1.86, 2.71, and 6.87 times higher, respectively, than those in the control at some time points (Figure 5Ba−d). *BoUCP1* expression was up-regulated about 1.6 times at day 5 in the control group and then dropped to the original level at the end. Compared with the control group at the same day, ATP treatment further increased the *BoUCP1* expression by 23.44% at day 3 ($P \leq 0.05$). DNP constantly maintained the *BoUCP1* transcription at relative lower levels which were 21.39 and 38.45% lower than those in the control at days 3 and 5, respectively (Figure 5Be). No differences of *BoUCP1* expression in the three groups at day 7 were found.

DISCUSSION

During the germination and sprouting of edible sprout seeds, complicated physiological activities result in marked changes of phenotypic traits and nutritive composition. Energy metabolism may play a crucial role in the dramatic variation. In this study, we tested the effects of energy states on the germination and sprouting changes by exogenous ATP and DNP. Also, the underlying energy metabolism during the development was preliminarily checked. Furthermore, the potential application of energy supply in preharvest sprouts production with higher quality was evaluated.

Rehydrated seed must quickly recover from a dormant state by a series of cellular events to get ready for the embryo emergence and seedling growth.[31] Results of germination and sprouting showed that, although exogenous ATP did not affect the GR of broccoli seeds at the end of germination (day 3) compared with control group, more germinated seeds were recorded after 1 day's germination (day 2) under ATP treatment (Figure 1Aa). The GI which statistically reflected the seed vigor and germination speed further confirmed the positive effect induced by ATP (Figure 1B). With the acceleration of the germination process, the positive regulation of ATP treatment on sprouting was also observed (Figure 1Ab). Higher levels of shoot length and fresh and dry weights were monitored. The underlying reason is that seed GI is positively related to seedling establishment.[32] Furthermore, the treatment of ATP inhibitor (DNP) resulted in significant oppositive effects on the germination and sprouting of broccoli seeds. All of these results indicated that energy metabolism plays a key role in this physiologic activity and that the active energy regulation could be an effective strategy to control the process.

Quality analysis revealed increased contents of general nutritional factors although some factors such as soluble sugar, anthocyanin, or total phenonics were maintained at similar or even lower levels at the end of sprouting (day 7) when compared to early sprouting (day 5; Figure 2). Various bioactive compounds are synthesized to form the cell components or regulate the physiological functions.[31] The nutritive components including soluble sugar and protein contents were improved by the exogenous ATP supply by different degrees. The plant pigments, chlorophyll, carotenoid, and anthocyanin, not only contribute to the color appearance and photosynthesis of sprouts but also exhibit anticarcinogenic and chemoprotective effects for human beings.[33] The pigments were abundantly synthesized especially during the sprouting stage (Figure 2c−e). A growing body of evidence indicates that ATP acts as an energy supplier and regulator for pigment biosynthesis such as chlorophyll.[34] Results showed that exogenous ATP treatment promoted the pigments formation,

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

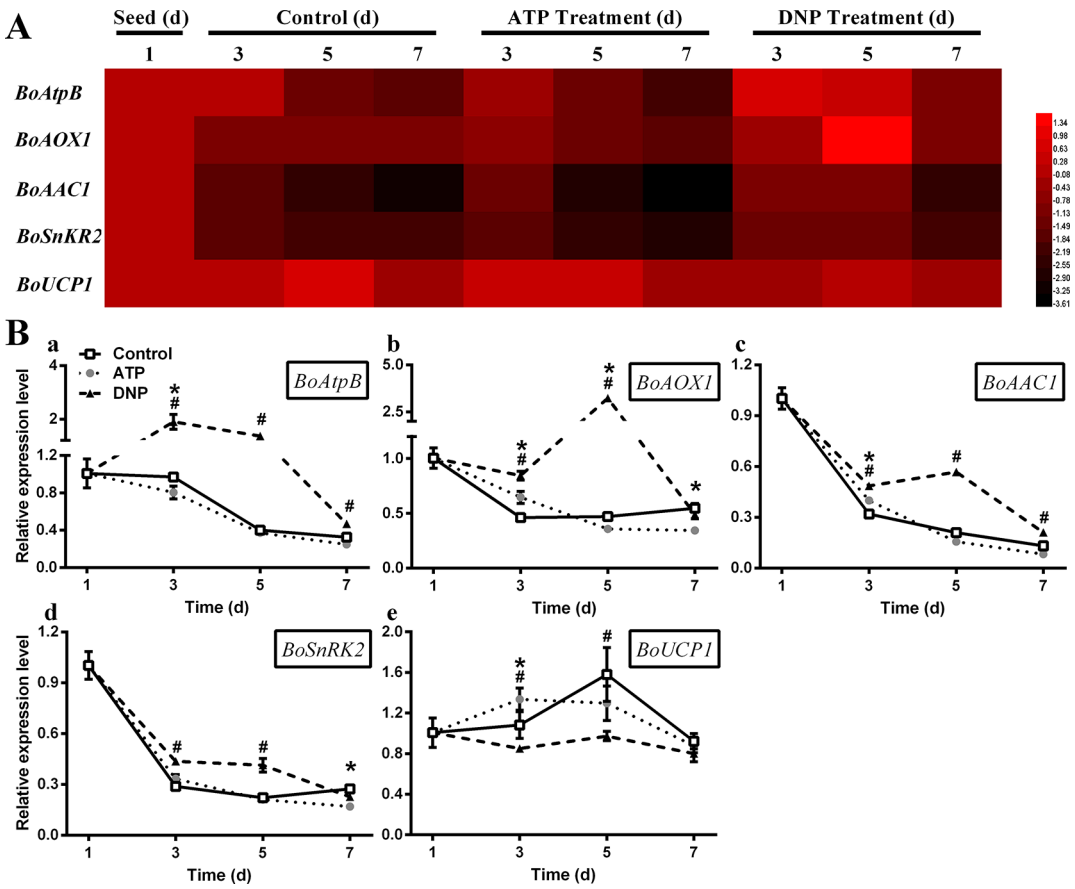


Figure 5. (A) Heatmap of energy-related genes under different treatments. (B) Effect on ATP and DNP on the relative expression levels of *BoAtpB*, *BoSnRK2*, *BoACC1*, *BoAOX1*, and *BoUCP1*. Note: the mark * (ATP treated group) or # (DNP treated group) indicate significant ($P \leq 0.05$) differences as compared to the control group at each production time.

and this may also help to explain the higher accumulation of biomass in the ATP treated group (Figure 1). Previous studies report the improvement of polyphenols in germination and sprouting processes of edible seeds. These secondary metabolites are effective nonenzymic antioxidants which balance the redox equilibrium in plant cells.[35] Our results were consistent with the previous reports that the TPCs were increased along with the germination and early sprouting progresses.[7−10] Furthermore, ATP stimulated the biological accumulation of total phenolics. Also, DNP treatment significantly decreased the concentrations of nutritional ingredients compared with those for the control group at the same test day which suggest that ATP is a pivotal factor in quality formation of broccoli sprouts.

Contents of antioxidant compounds in plants may influence the free radical scavenging capacities. Some studies confirm the relationship between phenolics contents and antioxidant capacities in different fruits and vegetables.[36−38] Thus, the AAs of methanolic extracts of broccoli seeds and sprouts and the linear correlation between the TPC and AAs were tested (Figure 3). The results showed similar changing trends by three test methods (Figure 3a−c) and were also consistent with TPC results (Figure 2f). Constantly increased TPCs and AAs were observed in the control group from days 1 to 5. ATP treatment induced higher phenolics contents and also exhibited elevated antioxidant capacities. Exogenous DNP decreased the contents of phenolics and AAs by different degrees. Correlation analysis further confirmed the positive relation between TPCs and AAs during the germination and sprouting of broccoli (Figure 3d). Significant ($P \leq 0.05$) and relative high linear correlation between TPCs and AAs tested by DPPH and FRAP assays (R^2 = 0.8677 and 0.7809, respectively) suggest that the TPC is a good indicator of in vitro AA. The lower (R^2 = 0.5768) but still statistically significant correlation between TPC and AA measured by the ABTS method indicated that some nonphenolic acids such as ascorbic acid might be involved in the ABTS radicals scavenging. Also, many kinds of flavonoids in plants also present high AAs.[39] Moure et al.[40] reported that the total flavonoid content was positive related to the AA determined by DPPH, ABTS, and FRAP methods.

To better understand the phenolics metabolism during sprouts production, the free phenolic acids extracted by methanol were measured in this study. It was found that chlorogenic and caffeic acids constituted higher concentrations in ungerminated seeds. This result is in accordance with the data obtained by Pająk et al.[28] Furthermore, with the sharp fall in chlorogenic acid contents during the growth progress, contents of caffeic acid were continuously increased (Table 2). Chlorogenic acid, a natural antioxidant associated with plant protection, is the hydroxycinnamoyl ester of quinic acid.[41] The decrease of chlorogenic acid at the early stage might contribute to the production of other complex phenolic compounds.[42] DNP notably slowed the chlorogenic acid degradation, and caffeic acid production suggested ATP is necessary for these processes. In addition, lower contents of phenolic acids

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

including P-coumaric, gallic, sinapic, and ferulic acids and flavonoids (kaempferol and quercetin) were also found in broccoli seeds and sprouts (Table 2). Different with our data, Pérez-Balibrea et al.[43] reported that ferulic and sinapic acids were the predominant free phenolic acids ranging from 18.9 to 35.2 mg 100 g^{-1} fresh weight in broccoli seeds and sprouts. The different results of phenolics in diverse reports may result from various factors such as extraction methods, broccoli species, growth environments, etc. Total content of phenolic acids exhibited a downtrend during the production which was not in agreement with TPC shown in Figure 2f. Phenolics constitute a large chemical family of secondary metabolites in plants with different forms such as ester, glycosides, and aglycones (free phenolic acids).[44] Thus, some other phenolic compounds might be synthesized and greatly contribute to the TPC. In addition, ATP treatment increased the TPA at the end of sprouting and DNP elevated the TPA at early stages. Because cholorogenic acid was the predominant component at early stages while caffeic acid mainly contributed to the TPA at end.

The energy status is a key factor in switching on seed germination and sprouting, and it was checked in this study. The results indicated that massive amounts of ATP, ADP, AMP, and total adenylate were produced for the physiological activities during germination (day 3) and kept at stable levels at the early sprouting stage (day 5). However, it should be noted that the energy charge was continuously decreased and at the end of sprouting (day 7), the energy homeostasis was disrupted (Figure 4). Based on the close relationship between energy status and quality formation, the decreased ATP might be responsible for the slowdown or even reduction of accumulation of quality factors (Figure 2) and antioxidant capacities (Figure 3) at the end of sprouts production (day 7). Similar with postharvest application of ATP treatments, preharvest supply of exogenous ATP effectively elevated the levels of ATP, ADP, AMP, total adenylate, and energy charge in germinated seeds and sprouts at some time points and effectively prevented the energy depletion at day 7. The DNP weakened the energy supply by reducing the contents of ATP, ADP, total adenylate, and energy charge which was also observed in postharvest longan fruits treated by DNP.[16] Chen et al.[15] reported that the ATP treatment effectively maintained the levels of chlorophyll, carotenoid, anthocyanin, and flavonoid in harvested longan fruits. Moreover, results of Yi et al.[18] showed the positive correlation between ATP level and antioxidant activity in litchi pericarp which indicated that ATP is participated in the synthesis of antioxidants such as phenolics. Our results were similar to the previous studies in postharvest field and further verified the positive physiological regulation function of ATP or energy status at early preharvest stage of crops production. Based on the present data, it could be concluded that exogenous ATP supply effectively improved the energy status of broccoli seeds and sprouts and subsequently promoted the growth progress and accumulation of nutritive factors such as antioxidant phenolics.

The transcriptional information on energy-related genes was checked to clarify the underlying energy metabolism during the germination and sprouting. Heatmap indicated that compared with the control group, exogenous ATP decreased levels of most energy-related genes at the end of the sprouting stage, while DNP induced higher transcriptions at early stages (Figure 5A). Detailed results showed that the *BoAtpB* was constantly down-regulated (Figure 5Ba) when the ECs were maintained at relative high levels (Figure 4e). ATP treatment lowered the expression level at day 3 which was also reported by Wang et al.[14] Moreover, the DNP-induced energy starvation significantly induced the *BoAtpB* expression. The results indicated that ATP synthase was regulated by feedback inhibition of energy status. Furthermore, recent studies reported that AtpB also acts as a pro-cell-death protein. The increase of *AtpB* expression level may indicate the initiation of the aging process.[45,46] It can help to explain the decreased expressions of *BoAtpB* during the early germination and sprouting. SnRK acts as the regulation center of energy metabolism in the SnRK pathway of plants. It can sense the energy depletion and inactivate the energy-consuming processes, trigger downstream *AtpB* transcription to maintain the energy homeostasis.[47] In the present study, *BoSnRK2* expression in the control group was decreased continuously, and a slight rise was observed at day 7 (Figure 5Bd) when EC was significantly decreased (Figure 4e). Similar with *BoAtpB*, ATP supply depressed the *BoSnRK2* expression at day 7 while DNP significantly induced its expression at days 3 and 5. Thus, the *BoAtpB* might be regulated directly by *BoSnRK2* in broccoli. AAC is involved in energy supply from mitochondria to cytosol and further to other organelles by catalyzing the counter-exchange between cytosolic ADP and matrix ATP.[48] *BoAAC1* exhibited similar changes in expression levels with *BoSnRK2* (Figure 5c,d) which was consistent with previous studies.[14]

AOX and UCP are two widespread energy dissipation systems in plants. As a terminal oxidase in the mitochondrial electron transport chain, AOX bypasses complexes III and IV regulated energy production by directly accepting electrons and transferring them to oxygen.[29] On the other hand, UCP reduces ATP yield by dissipating the respiratory chain based proton electrochemical gradient. Our results indicated that the two systems represented different performances under different energy status. *BoAOX1* was suppressed and *BoUCP1* was induced under sufficient energy status at germination and early sprouting stages. At the end of sprouting when energy started to deplete, expression of *BoAOX1* was slightly improved while *BoUCP1* was significantly suppressed. DNP induced energy depletion advanced and enhanced the responses, while exogenous ATP constantly depressed *BoAOX1* at days 5 and 7 but induced high levels of *BoUCP1* at day 3. Based on the transcriptional results, the *BoSnRK2*-mediated energy system was mainly regulated by the feedback inhibition of energy status. At the early stage of broccoli sprouts production, the expressions of *BoSnRK2* system including *BoSnRK2*, *BoAtpB*, *BoACC1* and *BoAOX1* were suppressed by high energy charge, and *BoUCP1* was the major ATP dissipation system. Energy imbalance at the end of production awakened the *BoSnRK2* energy supplement system, and *BoAOX1* increased.

In conclusion, this study investigated the energy-regulated nutrition during broccoli germination and sprouting. At germination and early sprouting stages, sufficient ATP was synthesized to maintain the energy charge which was involved in the germination progress and sprouts biomass accumulations. In addition, secondary metabolism based nutritional compositions and antioxidant capacities were significantly increased. The expression of the *BoSnRK2* pathway of energy metabolism including factors of *BoSnRK2*, *BoAtpB*, *BoACC1*, and *BoAOX1* was suppressed when the high level of EC was inhibited. *BoUCP1* was the major energy dissipation factor at this stage. At the end of sprouting (day 7), energy balance was

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

disrupted, and the energy-required quality and antioxidant accumulations were weakened. The *BoSnRK2* and *BoAOX1* were slightly activated. DNP-induced energy starvation further clarified the activation of the *BoSnRK2* pathway which resulted in a certain extent of energy homeostasis and reprogrammed sprout growth and metabolism (Figure 6). In all, exogenous

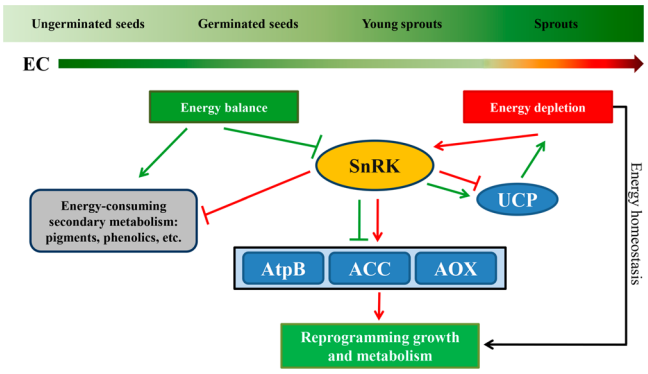


Figure 6. Synopsis of energy regulated broccoli germination and sprouting. Note: EC, energy charge; SnRK, sucrose nonfermenting-1-related kinase; UCP, uncoupling proteins; AtpB, ATP synthase β subunit; ACC, ADP/ATP carrier; AOX, alternative oxidase.

ATP supply was an effective strategy to keep the energy status of broccoli seeds and sprouts. It prevented the energy depletion by keeping the expression levels of *BoSnRK2* and related genes at a low level and further promoted the germination, sprouting, and accumulation of nutrition. Preharvest ATP treatment could be a potential strategy for producing the sprouts with high quality.

AUTHOR INFORMATION

Corresponding Author

*Tel: 65-6516 4695. Fax: 65-6775 7895. E-mail: chmynghs@nus.edu.sg.

ORCID

Hongshun Yang: 0000-0002-2965-4353

Funding

This study was funded by the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112), projects 31371851 and 31071617 supported by NSFC, Natural Science Foundation of Jiangsu Province (BK20141220), and an industry grant supported by Changzhou Qihui Management & Consulting Co., Ltd. (R-143-000-616-597).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AA, antioxidant activity; AtpB, ATP synthase β subunit; AOX, alternative oxidase; UCP, uncoupling proteins; AAC, ADP/ATP carrier; SnRK, sucrose nonfermenting-1-related kinase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; EC, energy charge; DNP, 2,4-dinitrophenol; DI, deionized; GR, germination rate; GI, germination index; TPTZ, 2,4,6-tri(2-pyridyl)-s-triazine; AMP, adenosine monophosphate; TPC, total phenolics content; TPA, total phenolic acids

REFERENCES

(1) Huang, K.; Nitin, N. Enhanced removal of *Escherichia coli* O157:H7 and *Listeria innocua* from fresh lettuce leaves using surfactants during simulated washing. *Food Control* **2017**, *79*, 207−217.

(2) Waje, C. K.; Jun, S. Y.; Lee, Y. K.; Moon, K. D.; Choi, Y. H.; Kwon, J. H. Seed viability and functional properties of broccoli sprouts during germination and postharvest storage as affected by irradiation of seeds. *J. Food Sci.* **2009**, *74*, C370−374.

(3) Li, Y. M.; Chan, H. Y. E.; Yao, X. Q.; Huang, Y.; Chen, Z. Y. Green tea catechins and broccoli reduce fat-induced mortality in *Drosophila melanogaster*. *J. Nutr. Biochem.* **2008**, *19*, 376−383.

(4) Moon, J. K.; Kim, J. R.; Ahn, Y. J.; Shibamoto, T. Analysis and anti-Helicobacter activity of sulforaphane and related compounds present in broccoli (*Brassica oleracea* L.) sprouts. *J. Agric. Food Chem.* **2010**, *58*, 6672−6677.

(5) Niether, W.; Smit, I.; Armengot, L.; Schneider, M.; Gerold, G.; Pawelzik, E. Environmental growing conditions in five production systems induce stress response and affect chemical composition of cocoa (*Theobroma cacao* L.) beans. *J. Agric. Food Chem.* **2017**, *65*, 10165−10173.

(6) Salaheen, S.; Nguyen, C.; Hewes, D.; Biswas, D. Cheap extraction of antibacterial compounds of berry pomace and their mode of action against the pathogen *Campylobacter jejuni*. *Food Control* **2014**, *46*, 174−181.

(7) Guo, X.; Li, T.; Tang, K.; Liu, R. H. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (*Vigna radiata*). *J. Agric. Food Chem.* **2012**, *60*, 11050−11055.

(8) Aguilera, Y.; Díaz, M. F.; Jiménez, T.; Benítez, V.; Herrera, T.; Cuadrado, C.; Martín-Pedrosa, M.; Martín-Cabrejas, M. A. Changes in nonnutritional factors and antioxidant activity during germination of nonconventional legumes. *J. Agric. Food Chem.* **2013**, *61*, 8120−8125.

(9) Duenas, M.; Hernández, T.; Estrella, I.; Fernández, D. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chem.* **2009**, *117*, 599−607.

(10) Wang, H.; Wang, J.; Guo, X.; Brennan, C. S.; Li, T.; Fu, X.; Chen, G.; Liu, R. H. Effect of germination on lignan biosynthesis, and antioxidant and antiproliferative activities in flaxseed (*Linum usitatissimum* L.). *Food Chem.* **2016**, *205*, 170−177.

(11) Zhang, N.; Zhang, H.; Sun, Q.; Cao, Y.; Li, X.; Zhao, B.; Wu, P.; Guo, Y. Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. *Sci. Rep.* **2017**, *7*, 503.

(12) Smiri, M.; Chaoui, A.; El Ferjani, E. Respiratory metabolism in the embryonic axis of germinating pea seed exposed to cadmium. *J. Plant Physiol.* **2009**, *166*, 259−269.

(13) Kramer, D. M.; Evans, J. R. The importance of energy balance in improving photosynthetic productivity. *Plant Physiol.* **2011**, *155*, 70−78.

(14) Wang, H.; Qian, Z.; Ma, S.; Zhou, Y.; Patrick, J. W.; Duan, X.; Jiang, Y.; Qu, H. Energy status of ripening and postharvest senescent fruit of litchi (*Litchi chinensis* Sonn.). *BMC Plant Biol.* **2013**, *13*, 55.

(15) Chen, M.; Lin, H.; Zhang, S.; Lin, Y.; Chen, Y.; Lin, Y. Effects of adenosine triphosphate (ATP) treatment on postharvest physiology, quality and storage behavior of longan fruit. *Food Bioprocess Technol.* **2015**, *8*, 971−982.

(16) Lin, Y.; Chen, M.; Lin, H.; Hung, Y. C.; Lin, Y.; Chen, Y.; Wang, H.; Shi, J. DNP and ATP induced alteration in disease development of Phomopsis longanae Chi-inoculated longan fruit by acting on energy status and reactive oxygen species production-scavenging system. *Food Chem.* **2017**, *228*, 497−505.

(17) Yao, F.; Huang, Z.; Li, D.; Wang, H.; Xu, X.; Jiang, Y.; Qu, H. Phenolic components, antioxidant enzyme activities and anatomic structure of longan fruit pericarp following treatment with adenylate triphosphate. *Sci. Hortic.* **2014**, *180*, 6−13.

(18) Yi, C.; Jiang, Y.; Shi, J.; Qu, H.; Xue, S.; Duan, X.; Shi, J.; Prasad, N. K. ATP-regulation of antioxidant properties and phenolics

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985

in litchi fruit during browning and pathogen infection process. *Food Chem.* **2010**, *118*, 42−47.
(19) Chen, L.; Zhou, Y.; He, Z.; Liu, Q.; Lai, S.; Yang, H. Effect of exogenous ATP on the postharvest properties and pectin degradation of mung bean sprouts (*Vigna radiata*). *Food Chem.* **2018**, *251*, 9−17.
(20) Yi, C.; Qu, H.; Jiang, Y.; Shi, J.; Duan, X.; Joyce, D.; Li, Y. ATP-induced changes in energy status and membrane integrity of harvested litchi fruit and its relation to pathogen resistance. *J. Phytopathol.* **2008**, *156*, 365−371.
(21) Baenas, N.; García-Viguera, C.; Moreno, D. A. Biotic elicitors effectively increase the glucosinolates content in *Brassicaceae* sprouts. *J. Agric. Food Chem.* **2014**, *62*, 1881−1889.
(22) Shi, Y.; Gao, L.; Wu, Z.; Zhang, X.; Wang, M.; Zhang, C.; Zhang, F.; Zhou, Y.; Li, Z. Genome-wide association study of salt tolerance at the seed germination stage in rice. *BMC Plant Biol.* **2017**, *17*, 92.
(23) Saengthongpinit, W.; Sajjaanantakul, T. Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. *Postharvest Biol. Technol.* **2005**, *37*, 93−100.
(24) Altisent, R.; Echeverria, G.; Graell, J.; Lopez, L.; Lara, I. Lipoxygenase Activity Is Involved in the Regeneration of Volatile Ester-Synthesizing Capacity after Ultra-Low Oxygen Storage of 'Fuji' Apple. *J. Agric. Food Chem.* **2009**, *57*, 4305−4312.
(25) Złotek, U.; Świeca, M.; Jakubczyk, A. Effect of abiotic elicitation on main health-promoting compounds, antioxidant activity and commercial quality of butter lettuce (*Lactuca sativa* L.). *Food Chem.* **2014**, *148*, 253−260.
(26) de Souza, V. R.; Pereira, P. A. P.; da Silva, T. L. T.; de Oliveira Lima, L. C.; Pio, R.; Queiroz, F. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chem.* **2014**, *156*, 362−368.
(27) Liu, Q.; Tan, C. S. C.; Yang, H.; Wang, S. Treatment with low-concentration acidic electrolysed water combined with mild heat to sanitise fresh organic broccoli (*Brassica oleracea*). *LWT - Food Sci. Technol.* **2017**, *79*, 594−600.
(28) Pająk, P.; Socha, R.; Gałkowska, D.; Rożnowski, J.; Fortuna, T. Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem.* **2014**, *143*, 300−306.
(29) Huang, Z.; Guo, L.; Wang, H.; Qu, H.; Ma, S.; Liu, Y.; Huang, H.; Jiang, Y. Energy status of kiwifruit stored under different temperatures or exposed to long-term anaerobic conditions or pure oxygen. *Postharvest Biol. Technol.* **2014**, *98*, 56−64.
(30) Chen, L.; Yang, Y.; Liu, C.; Zheng, Y.; Xu, M.; Wu, N.; Sheng, J.; Shen, L. Characterization of WRKY transcription factors in *Solanum lycopersicum* reveals collinearity and their expression patterns under cold treatment. *Biochem. Biophys. Res. Commun.* **2015**, *464*, 962−968.
(31) Nonogaki, H.; Bassel, G. W.; Bewley, J. D. Germination—Still a mystery. *Plant Sci.* **2010**, *179*, 574−581.
(32) Hu, X. W.; Wu, Y. P.; Ding, X. Y.; Zhang, R.; Wang, Y. R.; Baskin, J. M.; Baskin, C. C. Seed dormancy, seedling establishment and dynamics of the soil seed bank of *Stipa bungeana* (Poaceae) on the Loess Plateau of Northwestern China. *PLoS One* **2014**, *9*, e112579.
(33) Lee, J.; Hwang, Y. S.; Lee, J. D.; Chang, W. S.; Choung, M. G. Metabolic alterations of lutein, β-carotene and chlorophyll a during germination of two soybean sprout varieties. *Food Chem.* **2013**, *141*, 3177−3182.
(34) Zhang, D.; Li, Y.; Zhang, X.; Zha, P.; Lin, R. The SWI2/SNF2 chromatin-remodeling ATPase BRAHMA regulates chlorophyll biosynthesis in *Arabidopsis*. *Mol. Plant* **2017**, *10*, 155−167.
(35) Sharma, P.; Jha, A. B.; Dubey, R. S.; Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *2012*, 1−26.
(36) Zhang, J.; Yang, H. Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactuca sativa* Var. *Crispa* L). *Food Control* **2017**, *72*, 20−26.
(37) Fu, C.; Yang, X.; Lai, S.; Liu, C.; Huang, S.; Yang, H. Structure, antioxidant and α-amylase inhibitory activities of longan pericarp proanthocyanidins. *J. Funct. Foods* **2015**, *14*, 23−32.
(38) Feng, X.; Ng, V. K.; Mikš-Krajnik, M.; Yang, H. Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. *Food Bioprocess Technol.* **2017**, *10*, 89−102.
(39) Lu, Y.; Joerger, R.; Wu, C. Study of the chemical composition and antimicrobial activities of ethanolic extracts from roots of *Scutellaria baicalensis* Georgi. *J. Agric. Food Chem.* **2011**, *59*, 10934−10942.
(40) Moure, A.; Franco, D.; Sineiro, J.; Domínguez, H.; Núñez, M. a. J.; Lema, J. M. Antioxidant activity of extracts from *Gevuina avellana* and *Rosa rubiginosa* defatted seeds. *Food Res. Int.* **2001**, *34*, 103−109.
(41) Karaköse, H.; Jaiswal, R.; Deshpande, S.; Kuhnert, N. Investigation of the photochemical changes of chlorogenic acids induced by ultraviolet light in model systems and in agricultural practice with *Stevia rebaudiana* cultivation as an example. *J. Agric. Food Chem.* **2015**, *63*, 3338−3347.
(42) Ti, H.; Zhang, R.; Zhang, M.; Li, Q.; Wei, Z.; Zhang, Y.; Tang, X.; Deng, Y.; Liu, L.; Ma, Y. Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. *Food Chem.* **2014**, *161*, 337−344.
(43) Pérez-Balibrea, S.; Moreno, D. A.; García-Viguera, C. Genotypic effects on the phytochemical quality of seeds and sprouts from commercial broccoli cultivars. *Food Chem.* **2011**, *125*, 348−354.
(44) Ross, K.; Beta, T.; Arntfield, S. A comparative study on the phenolic acids identified and quantified in dry beans using HPLC as affected by different extraction and hydrolysis methods. *Food Chem.* **2009**, *113*, 336−344.
(45) Chivasa, S.; Tomé, D. F.; Hamilton, J. M.; Slabas, A. R. Proteomic analysis of extracellular ATP-regulated proteins identifies ATP synthase β-subunit as a novel plant cell death regulator. *Mol. Cell. Proteomics* **2011**, *10*, M110.003905.
(46) Martinez-Cruz, O.; Garcia-Carreño, F.; Robles-Romo, A.; Varela-Romero, A.; Muhlia-Almazan, A. Catalytic subunits atpα and atpβ from the Pacific white shrimp *Litopenaeus vannamei* F_OF_1 ATP-synthase complex: cDNA sequences, phylogenies, and mRNA quantification during hypoxia. *J. Bioenerg. Biomembr.* **2011**, *43*, 119−133.
(47) Hulsmans, S.; Rodriguez, M.; De Coninck, B.; Rolland, F. The SnRK1 energy sensor in plant biotic interactions. *Trends Plant Sci.* **2016**, *21*, 648−661.
(48) Klingenberg, M. The ADP and ATP transport in mitochondria and its carrier. *Biochim. Biophys. Acta, Biomembr.* **2008**, *1778*, 1978−2021.

DOI: 10.1021/acs.jafc.8b00466
J. Agric. Food Chem. 2018, 66, 6975−6985