



Article

Study of the Effects of Spraying Non-Bagging Film Agent on the Contents of Mineral Elements and Flavonoid Metabolites in Apples

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Abstract: There has been growing interest in examining the potential of non-bagging patterns due to the decline of fruit inner quality and the increase in labor force cost and ecological pollution. Spraying a non-bagging film agent is an important method for non-bagging cultivation. This paper aims to study the effects of non-bagging film agents on the contents of mineral elements and flavonoid metabolites in apple fruits and determine the feasibility of this method. Fuji apples were used as the sample material and treated individually with two non-bagging film agents, namely, humic acid film (ABM) and Pirrio calcium film (CAM). Also, two control groups, namely, the clear water spraying without bagging group (CK) and the bagging group (TCK), were set in this study to measure the contents of mineral elements and flavonoid metabolites in these apples. Compared with those two control groups, the spraying treatment groups with two kinds of non-bagging film agents present a significant difference between their total contents of mineral elements, with the total content of mineral elements of apples in the ABM treatment group being 1.36 times the content of apples in the CK group. In terms of the flavonoid metabolites, only Astragalin, Tiliroside, Homoplantagin, Phlorizin, Apigenin, Hesperidin, Oroxin A, and Kaempferol present significant differences in their proportions in apples, and there are no significant differences among the proportions of other compounds. Individual spraying of two kinds of non-bagging film agents can significantly increase the total contents of mineral elements in apples, with slight effects on the contents of flavonoid metabolites in these fruits. Therefore, both film agents can be used for cultivating Fuji apples.



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Keywords: apples; mineral elements; flavonoid metabolites

1. Introduction

Apple (*Malus domestica*), one of the most widely cultivated tree fruits in many regions of the world, is the most important fruit and is liked by all classes of people due to its pleasant taste and established nutritional and economical value. It is a rich source of antioxidant compounds, carbohydrates, essential minerals, and dietary fiber [1–3]. China is the largest apple-producing and -consuming country in the world [4].

The commonly used fruit bagging material in the current market is generally the film-coated paper bag. With good effects on water retention, freshness preservation, and weight increase, this bagging material can improve the appearance of fruits and their market value at the same time. However, such a cultivation method has changed the growth and development environments of apples, thus leading to problems affecting fruit quality, such

as reduced contents of phenolic compounds, diminished flavor quality, and some other problems [5–7]. In recent years, there has been growing interest in examining the potential of non-bagging patterns due to the decline of fruit inner quality and the increase in labor force cost and ecological pollution. It has been recognized that the bagging-free cultivation pattern is an inevitable trend in the apple industry development in China [8].

Spraying non-bagging film agents is an important method for practicing the non-bagging cultivation of fruits, with non-bagging film agents specifically used in the surface maintenance of fruits during development. With the spraying of film agent, a layer of “soft bi-layer biofilm” with a thickness of about 5–10 nanometers can be quickly formed, covering the surface layer of the fruit. Meanwhile, gaps between membrane molecules of the layer can allow the free passing-through of oxygen, carbon dioxide, and sunlight, thus providing all-around protection for preventing pesticides and pests from contaminating and harming plants.

At present, there are few studies on the influence of spraying non-bagging film agents on the fruit quality of apples. Under such a circumstance, this study has measured the contents of forty-two mineral elements and flavonoid metabolites in apple fruits with the assistance of an inductively coupled plasma emission spectrometer (ICP-MS). With Fuji apples taken as the research object, two control groups of clear water treatment and bagging treatment, as well as experimental treatment groups with individual spraying of two kinds of non-bagging film agents, were set in this study to analyze the contents of mineral elements and flavonoid metabolites in fruits under these four different treatment methods. Also, this study has explored the influences of spraying non-bagging film agents on the contents of mineral elements and flavonoid metabolites in Fuji apple fruits, with the study results providing basic data support for the further application of non-bagging cultivation.

2. Materials and Methods

2.1. Analytical Objects

A total of four test groups, namely, the clear water control group (CK), bagging treatment group (TCK), non-bagging humic acid film agent treatment group (ABM), and non-bagging Piriol calcium film agent treatment group (CAM), were designed in this study. Upon the removal of their fuzz, apples in the clear water control group, ABM group, and CAM group were sprayed with clear water and film agents correspondingly, with a spraying frequency of once every month for a consecutive period of three months. After the harvest of apple fruits, they were packed in cartons and delivered to the laboratory on the same day. Then, ten apples with similar shapes, sizes, and colors were picked from twenty apple samples under each treatment to investigate the differences among the physical and chemical indexes, mineral element contents, and flavonoid metabolite contents of apple fruits treated with different methods.

This study analyzed the physicochemical indicators such as sugar and acid and determined the contents of trace elements using a fresh sample; for mineral elements, a dried sample was used.

2.2. Instruments and Reagents

The instruments used for samples' preparation and analysis include: a microwave digestion system (MARS 6, CME, New York, NY, USA), an ICP-MS inductively coupled plasma mass spectrometer (ELAN DRC-e, Perkin Elmer, Waltham, MA, USA), an ultrapure water manufacturing system (Milli Plus 2150, MILLIPORE, Burlington, MA, USA), and a 6875D fully-automatic frozen grinder (Retsch, Haan, Germany). Flavonoids contents were detected by MetWare based on the AB Sciex QTRAP 6500 LC-MS/MS platform (Singapore, USA).

The reagents used for samples' preparation and analysis include: mixed standards of Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Gd, Hg, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, Pr, Rb, Sb, Sc, Sm, Sn, Sr, Ti, Th, U, V, Fe, Zn, P, Ru, Au, Ga, Ca, Zr, and Pb ($1000 \text{ mg} \cdot \text{L}^{-1}$), provided

by the National Institute of Metrology China, and prepared to the required concentration when used.

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). MilliQ water (Millipore, Molsheim, France) was used in all experiments. Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the standards were purchased from MCE (MedChemExpress, Shanghai, China). The stock solutions of standards were prepared at the concentration of $10 \text{ mmol} \cdot \text{L}^{-1}$ in 70% MeOH. All stock solutions were stored at -20°C . The stock solutions were diluted with 70% MeOH to working solutions before analysis.

HNO_3 , HClO_4 , and other chemicals used were of standard analytical grade. Deionized and distilled water was used throughout.

2.3. Measurement of Physical and Chemical Indexes

A 1% electronic balance was used to measure the single-fruit weight, a pointer-type fruit hardness tester was applied to measure the hardness of apples, and a Brix-acidity meter was used to measure the soluble solid contents and titratable acidity of apples.

2.4. Determination of Mineral Elements

Based on the method proposed by Liu et al. [9], a slightly modified analysis of inductively coupled plasma mass spectrometry was carried out in this study with the following specific operation steps:

- (1) Dissolving the samples using the microwave digestion method, with the specific operation steps as follows: Weigh 0.5 g of *Lycium barbarum* sample (with a precision of 0.0001 g), and put it in a microwave digestion tube. After the addition of 10 mL of nitric acid, let the sample stand at room temperature for 3 h, and then put it in a microwave digestion instrument for sample digestion. Select temperature control to let the temperature rise to 120°C in 5 min, and keep the temperature there for 10 min. Then, let the temperature rise to 150°C in 5 min and keep it there for 20 min. After that, let the temperature further rise to 185°C in 5 min and keep it there for 30 min. After the completion of digestion, the sample is cooled. Then, gently unscrew the lid and place the microwave digestion tube on an acid-driven processor to perform acid removal at 120°C for 2 h. Then, cool the sample to room temperature and wash it with ultra-pure water in a test tube with scale of 25 mL. After that, dilute it with ultra-pure water to volume and shake the solution well. Meanwhile, prepare a reagent blank.
- (2) Measure the contents of elements in apple fruits through the ICP-MS method under the following specific working conditions: Generator power: 1300 W; Atomizer flow rate: $0.95 \text{ L} \cdot \text{min}^{-1}$; Plasma torch cooling gas flow rate: $17.0 \text{ L} \cdot \text{min}^{-1}$; Auxiliary device flow rate: $1.20 \text{ L} \cdot \text{min}^{-1}$; Detector analog stage voltage: -2350 V ; Ion lens voltage: 6.00 V.

All samples were analyzed through the ICP-MS method, with each sample analyzed in duplicate. The working curve corresponding to each element was plotted, with its specific linear equation and correlation coefficient listed in Table 1. All correlation coefficients R^2 calculated are higher than 0.99, indicating good linear regressions of standard solution concentrations and absorption peak areas and the feasibility of using these curves to quantify the contents of mineral elements.

2.5. Metabolites Extraction

The sample was freeze-dried, ground into powder (30 Hz, 1.5 min), and stored at -80°C until needed. In total, 20 mg powder was weighted and extracted with 0.5 mL 70% methanol. In total, 10 μL internal standard ($4000 \text{ nmol} \cdot \text{L}^{-1}$) was added into the extract as an internal standard (IS) for the quantification. The extract was sonicated for 30 min and centrifuged at $12,000 \times g$ under 4°C for 5 min. The supernatant was filtered through a $0.22 \mu\text{m}$ membrane filter for further LC-MS/MS analysis.

Table 1. Influences of different treatment methods on the physical and chemical indexes of fruits.

Treatment	Single Fruit Weight (g)	Hardness (kg·cm ⁻²)	Soluble Solid Content (%)	Titrateable Acid Content (%)
CK	231 ± 43.8 ^b	8.48 ± 0.82 ^a	19.4 ± 1.80 ^a	0.35 ± 0.10 ^b
TCK	247 ± 44.2 ^b	9.17 ± 0.87 ^a	13.7 ± 1.96 ^a	0.35 ± 0.13 ^b
ABM	250 ± 30.7 ^a	9.25 ± 1.16 ^a	16.6 ± 1.34 ^a	0.46 ± 0.19 ^a
CAM	208 ± 18.7 ^b	8.86 ± 1.03 ^a	17.3 ± 1.38 ^a	0.41 ± 0.05 ^{ab}

Values with different letters in the same column (a,b) are significantly different ($p < 0.05$) from each other. Values are given as the mean ± standard deviation.

2.6. UPLC-ESI-MS/MS Analysis

The sample extracts were analyzed using a UPLC-ESI-MS/MS system. The analytical conditions were as follows: UPLC column, Waters ACQUITY UPLC HSS T3 C18 (1.8 µm, 100 mm × 2.1 mm i.d.); solvent system, water with 0.05% formic acid (A), acetonitrile with 0.05% formic acid (B). The gradient elution program was set as follows: 0–1 min, 10–20% B; 1–9 min, 20–70% B; 9–12.5 min, 70–95% B; 12.5–13.5 min, 95% B; 13.5–13.6 min, 95–10% B; 13.6–15 min, 10% B. The flow rate was set at 0.35 mL·min⁻¹ and the temperature was set at 40 °C. The injection volume is 2 µL.

The ESI source operation parameters were as follows: ion source, ESI +/−; source temperature 550 °C; ion spray voltage (IS) 5500 V (positive), −4500 V (negative); curtain gas (CUR) was set at 35 psi, respectively. Flavonoids were analyzed using scheduled multiple reaction monitoring (MRM).

2.7. Data Processing and Statistical Analysis

The mean value (MEAN) and standard deviation (SD) of the traits were calculated using Microsoft Excel 2016 and were statistically analyzed using IBM SPSS Statistics 26.0 software. Independent-samples *t*-tests were used to determine the significance of differences among samples at a level of 0.05. Values were reported as means ± standard deviation (SD) from triplicate experiments. Mass spectrometric data conducted in MultiQuant 3.0.3.

3. Results

3.1. Effects of Non-Bagging Film Agent Treatment on the Physical and Chemical Indexes of Fruits

Single-fruit weight, hardness, soluble solids, and total acidity are important indicators for evaluating apple quality. We found that there is a significant difference between the ABM treatment group, which has the largest single-fruit weight, and the CAM treatment group, which has the smallest single-fruit weight. However, in terms of the single-fruit weight, the control groups are not significantly different from the CAM treatment group. Therefore, it can be inferred that the single-fruit weights of apples in the ABM treatment group have significantly increased (Table 1). Also, it was found that in terms of apple hardness, there was no significant difference among all treatment groups (Table 1). An analysis of the TSS contents shows that there was no significant difference among all treatment groups, which shows the insignificant influence on the TSS of apples. In terms of the influence on titrateable acid, there were significant differences among different treatment groups, with the titrateable acid contents in the non-bagging film agent treatment groups being significantly higher than those contents in the control groups ($p < 0.05$). In summary, the treatment of non-bagging film agents increases the single-fruit weights of apples and improves the accumulation of titrateable acid.

3.2. Effects of Non-Bagging Film Agent Treatment on the Contents of Mineral Elements of Fruits

Mineral element content is one way to measure fruit quality traits, as mineral elements are closely related to fruit size, pulp hardness, and soluble solids, and play important roles in fruit disease resistance storage resistance, and maintaining good quality and flavor [10,11]. Mineral elements are also very important nutrients that are essential for healthy human growth and development [12].

Among all treatment groups, the contents of P, Mg, Ca, and Na are significantly higher than those contents of other trace elements, with their relative ranges between $0.000482 \text{ mg} \cdot \text{kg}^{-1}$ and $1789 \text{ mg} \cdot \text{kg}^{-1}$ (Table 2). Among all these elements, the content of element P, which ranges from $1185 \text{ mg} \cdot \text{kg}^{-1}$ to $1789 \text{ mg} \cdot \text{kg}^{-1}$, is the highest. The contents of the elements Ca and Na are in the same order of magnitude as the content of the element Mg ($109\text{--}417 \text{ mg} \cdot \text{kg}^{-1}$). Also, the contents of elements Al, B, and V are in the same order of magnitude as the content of the element Fe ($11.5\text{--}154 \text{ mg} \cdot \text{kg}^{-1}$), and the contents of the elements As, Cr, Cu, Mn, Rb, Sr, Zn, and Ga are in the same order of magnitude ($1.4\text{--}6.06 \text{ mg} \cdot \text{kg}^{-1}$), with the contents of all of the other elements being lower than $1 \text{ mg} \cdot \text{kg}^{-1}$ (Table 2). An analysis of correlation variance shows that among those forty-one elements of apple fruits, there are no significant differences among the contents of fifteen elements (B, Cu, Mg, Mo, Pr, Sb, Sm, Sn, Ti, Th, Ru, Ga, Zr, and Ca) ($p > 0.05$), indicating that there are no significant differences among the apple fruits' absorption capacities of these elements.

Table 2. Effects of different treatment methods on the contents of mineral elements of fruits ($\text{mg} \cdot \text{kg}^{-1}$).

Element	CK	TCK	ABM	CAM
Al	38.4 ± 5.7^b	29.7 ± 7.5^b	154 ± 51^a	43.4 ± 9.7^b
As	2.36 ± 0.15^a	2.35 ± 0.14^a	2.1 ± 0.20^{ab}	1.97 ± 0.23^b
B	69.6 ± 8.01^a	73.4 ± 9.08^a	77.2 ± 4.15^a	80.8 ± 5.86^a
Ba	0.91 ± 0.0884^c	2.48 ± 0.5922^b	1.24 ± 0.2521^c	4.86 ± 0.9407^a
Be	0.0015 ± 0.001^b	0.0065 ± 0.003^{ab}	0.0105 ± 0.007^a	0.0052 ± 0.001^{ab}
Bi	0.000652 ± 0.0002^b	0.000564 ± 0.0002^b	0.0013 ± 0.0004^a	0.000482 ± 0.0004^b
Cd	0.00116 ± 0.0001^b	0.00161 ± 0.0007^b	0.00151 ± 0.0006^b	0.00251 ± 0.0007^a
Ce	0.0166 ± 0.0038^b	0.021 ± 0.0074^{ab}	0.0274 ± 0.0047^a	0.014 ± 0.0073^b
Co	0.0228 ± 0.0067^b	0.0265 ± 0.0047^{ab}	0.0362 ± 0.0096^a	0.0344 ± 0.0047^a
Cr	1.86 ± 0.24^b	2.17 ± 0.37^b	2.61 ± 0.28^a	1.91 ± 0.28^b
Cs	0.0085 ± 0.0015^b	0.0180 ± 0.0042^a	0.0122 ± 0.0035^b	0.0085 ± 0.0014^b
Cu	1.69 ± 0.08^a	2.17 ± 0.31^a	2.15 ± 0.57^a	1.78 ± 0.28^a
Gd	0.000993 ± 0.0002^b	0.00125 ± 0.0003^b	0.00217 ± 0.0006^a	0.00142 ± 0.0003^b
Hg	0.0039 ± 0.0008^b	0.0024 ± 0.0003^b	0.0055 ± 0.0001^b	0.0708 ± 0.0138^a
Li	0.831 ± 0.065^a	0.5213 ± 0.077^b	0.6255 ± 0.17^b	0.3773 ± 0.11^c
Mg	363 ± 24.7^a	361 ± 39.3^a	417 ± 47^a	405 ± 37.9^a
Mn	1.66 ± 0.19^d	2.25 ± 0.31^c	3.44 ± 0.53^a	2.69 ± 0.23^b
Mo	0.177 ± 0.033^a	0.1562 ± 0.023^a	0.1197 ± 0.016^a	0.1772 ± 0.055^a
Na	109 ± 5.18^b	126 ± 16.7^b	257 ± 12.1^a	130 ± 18^b
Nb	0.0116 ± 0.0057^a	0.0058 ± 0.0026^b	0.0117 ± 0.0035^a	0.0047 ± 0.0011^b
Nd	0.0069 ± 0.001^b	0.0059 ± 0.0005^{bc}	0.0086 ± 0.0011^a	0.0053 ± 0.0014^c
Ni	0.3374 ± 0.028^b	0.3277 ± 0.107^b	0.3831 ± 0.035^b	0.5525 ± 0.131^a
Pr	0.0024 ± 0.0009^a	0.0025 ± 0.0009^a	0.0034 ± 0.0005^a	0.0033 ± 0.0014^a
Rb	2.81 ± 0.53^b	4.98 ± 0.87^a	4.4 ± 0.97^a	4.18 ± 0.24^a
Sb	0.0136 ± 0.0027^a	0.0087 ± 0.0026^a	0.0114 ± 0.0018^a	0.015 ± 0.0076^a
Sc	0.0927 ± 0.013^a	0.059 ± 0.0029^b	0.0815 ± 0.035^{ab}	0.0527 ± 0.014^b
Sm	0.0013 ± 0.0007^a	0.0016 ± 0.0006^a	0.0014 ± 0.0002^a	0.0016 ± 0.0006^a
Sn	0.0618 ± 0.012^a	0.0524 ± 0.0077^a	0.0542 ± 0.0065^a	0.0591 ± 0.014^a
Sr	1.74 ± 0.23^c	6.06 ± 1.36^a	4.27 ± 0.83^b	1.4 ± 0.25^c
Ti	1.2 ± 0.26^a	1.33 ± 0.38^a	0.76 ± 0.09^a	0.93 ± 0.54^a
Th	0.0022 ± 0.0013^a	0.0024 ± 0.0026^a	0.0049 ± 0.0018^a	0.0022 ± 0.0008^a
U	0.0061 ± 0.0006^a	0.0044 ± 0.0008^{ab}	0.0049 ± 0.0006^b	0.0036 ± 0.0007^c
V	15.6 ± 1.79^a	16.6 ± 1.88^a	16.8 ± 2.74^a	11.5 ± 0.66^b
Fe	38.4 ± 8.2^{ab}	32.1 ± 4.9^b	45.7 ± 7.8^a	40.7 ± 7.6^{ab}
Zn	4.38 ± 0.41^{ab}	3.65 ± 0.45^b	4.39 ± 0.62^{ab}	5.07 ± 1.11^a
P	1185 ± 134^c	1395 ± 215^{bc}	1567 ± 175^{ab}	1789 ± 219^a
Ru	0.038 ± 0.0089^a	0.0352 ± 0.013^a	0.0427 ± 0.023^a	0.0288 ± 0.0077^a
Au	0.0333 ± 0.0068^a	0.0165 ± 0.0023^b	0.0138 ± 0.0018^b	0.0171 ± 0.0008^b
Ga	2.02 ± 0.44^a	2.3 ± 0.68^a	2.38 ± 0.78^a	2.87 ± 0.78^a
Zr	0.0219 ± 0.0039^a	0.0251 ± 0.013^a	0.0276 ± 0.0017^a	0.0227 ± 0.0049^a
Ca	242 ± 47^a	250 ± 28^a	262 ± 7.3^a	272 ± 32.9^a
Pb	0.027 ± 0.0054^{ab}	0.0167 ± 0.0055^b	0.0298 ± 0.0047^{ab}	0.0386 ± 0.018^a

Values with different letters in the same line (a–c) are significantly different ($p < 0.05$) from each other. Values are given as the mean $\pm$ standard deviation.

Among those forty-two elements mentioned above, thirteen elements (Al, As, Hg, Pb, Cd, Cr, Ni, Sn, Mo, V, Ti, Sb, and Ba) are harmful elements. The contents of the Pb and Cd elements of apples in each treatment group are all below the limits stipulated in the National Food Safety Standard—Maximum Levels for Contaminants in Food (GB 2762-2022) [13] on Pb ($<0.1 \text{ mg}\cdot\text{kg}^{-1}$) and Cd ($<0.05 \text{ mg}\cdot\text{kg}^{-1}$) contents in fruits. Among these thirteen elements, in this study, only high contents of Al elements were measured in apples. Also, the contents of the Al element varied significantly from group to group, with the Al element contents of apples in the ABM treatment group being 5.2 times those contents of apples in the TCK group. However, no relevant limit on the Al element is stipulated in the standard described above.

The results indicate that apples in the ABM treatment group present the highest total mineral element content, followed by the apples in the CAM treatment group, and the apples in the TCK and CK groups present the lowest total mineral element content (Figure 1). Also, the total mineral element content of apples in the ABM treatment group is 1.36 times the content of apples in the CK group. To sum up, the treatment of the non-bagging film agent will not change the composition of mineral elements in apples but will increase their total contents of mineral elements.

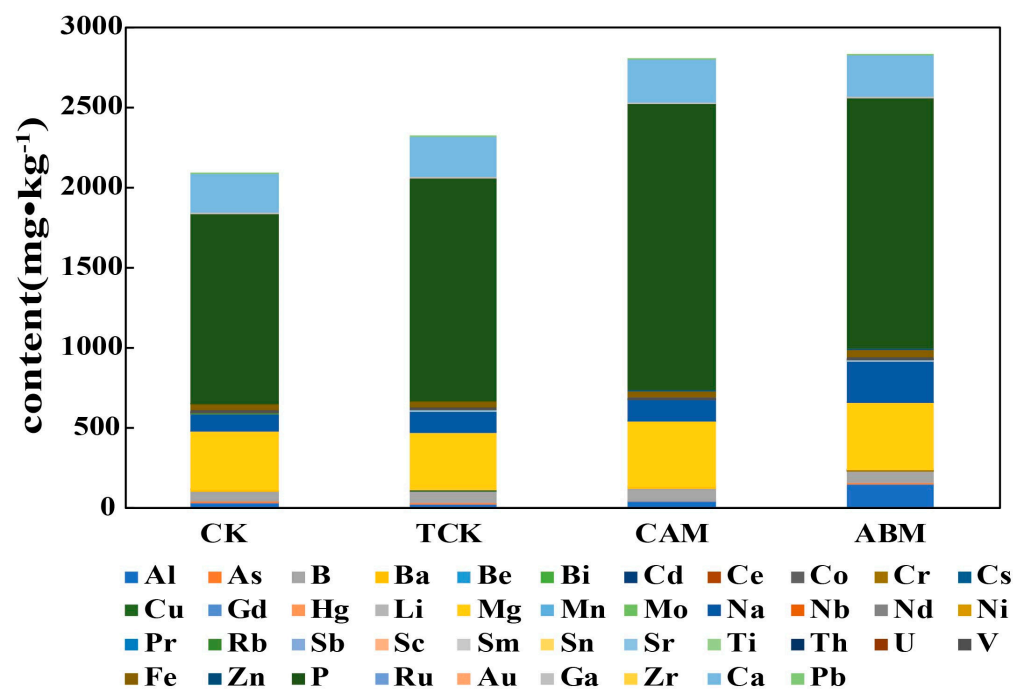


Figure 1. Influences of different treatment methods on the total mineral element content of apples.

3.3. Effects of Non-Bagging Film Agent Treatment on the Contents of Flavonoid Metabolites in Apples

In this study, a total of 204 types of metabolites were selected for testing. Some metabolite types had no content detected in apples, while some other metabolite types with relatively low contents detected in apples were not counted. Therefore, this study has performed a comprehensive analysis on a total of 39 metabolite types that were detected in apples with relatively high contents. From Table 3, it can be seen that flavonoid metabolites have a rich presence in apple fruits, with relatively great variations in contents ranging from 0.002 to $466 \text{ nmol}\cdot\text{g}^{-1}$. Followed by Quercitrin, Astragalin, Rutin, Avicularin, Calycosin-7-O- β -D-glucoside, and Hyperoside, whose contents fall into a range between $1 \text{ nmol}\cdot\text{g}^{-1}$ and $100 \text{ nmol}\cdot\text{g}^{-1}$, (-)-Epicatechin, (-)-Catechin, and Phlorizin have the highest contents, which are all above $100 \text{ nmol}\cdot\text{g}^{-1}$. Also, it shows that there are relatively small differences among the influences of different treatment methods on the contents of 39 metabolites. An analysis of correlation variance indicates that only Astragalin, Tiliroside, Homoplantagin, and

Phlorizin, Apigen, Hesperidin, Oroxin A, and Kaempferol present significant differences in their contents in apples, with no significant content differences among other metabolites, indicating insignificant influences of the non-bagging film agent treatment methods on the apple contents of flavonoid metabolites.

Table 3. Influences of different treatment methods on the contents of flavonoid metabolites in fruits (nmol·g^{−1}).

Compounds	CK	TCK	ABM	CAM
Astilbin	0.165 ± 0.05 ^a	0.088 ± 0.01 ^a	0.128 ± 0.03 ^a	0.183 ± 0.04 ^a
Pinocembrin	0.302 ± 0.28 ^a	0.219 ± 0.18 ^a	0.260 ± 0.25 ^a	0.276 ± 0.22 ^a
Quercitrin	54.7 ± 4.9 ^a	44.9 ± 35.9 ^a	41.3 ± 12.8 ^a	50.1 ± 7.02 ^a
Narcissin	0.025 ± 0.008 ^a	0.022 ± 0.008 ^a	0.015 ± 0.004 ^a	0.015 ± 0.004 ^a
Astragalin	2.28 ± 0.73 ^a	0.372 ± 0.20 ^b	0.899 ± 0.05 ^b	1.48 ± 0.76 ^{ab}
Tiliroside	0.046 ± 0.006 ^c	0.062 ± 0.02 ^{bc}	0.084 ± 0.02 ^{ab}	0.108 ± 0.01 ^a
(-)-Epicatechin	358 ± 60 ^a	466 ± 59 ^a	461 ± 118 ^a	450 ± 46.4 ^a
(-)-Catechin gallate	0.107 ± 0.03 ^a	0.119 ± 0.02 ^a	0.146 ± 0.01 ^a	0.090 ± 0.02 ^a
Isosakuranetin	0.009 ± 0.005 ^a	0.005 ± 0.005 ^a	0.009 ± 0.008 ^a	0.012 ± 0.006 ^a
Apigenin 7-glucoside	0.033 ± 0.017 ^a	0.008 ± 0.002 ^a	0.008 ± 0.005 ^a	0.025 ± 0.01 ^a
Tectochrysin	0.013 ± 0.008 ^a	0.015 ± 0.01 ^a	0.021 ± 0.02 ^a	0.012 ± 0.008 ^a
Homoplantagin	0.078 ± 0.01 ^a	0.045 ± 0.005 ^b	0.056 ± 0.008 ^b	0.051 ± 0.009 ^b
6-Hydroxyflavone	0.020 ± 0.003 ^a	0.016 ± 0.003 ^a	0.020 ± 0.003 ^a	0.018 ± 0.001 ^a
Naringenin-7-glucoside	0.526 ± 0.01 ^a	0.635 ± 0.15 ^a	0.950 ± 0.24 ^a	0.939 ± 0.15 ^a
Phloretin	0.058 ± 0.009 ^a	0.065 ± 0.004 ^a	0.070 ± 0.02 ^a	0.077 ± 0.017 ^a
Spiraeoside	0.039 ± 0.009 ^a	0.022 ± 0.019 ^a	0.024 ± 0.015 ^a	0.030 ± 0.008 ^a
Quercimeritrin	0.562 ± 0.16 ^a	0.525 ± 0.59 ^a	0.423 ± 0.11 ^a	0.404 ± 0.11 ^a
Isorhamnetin 3-O-glucoside	0.149 ± 0.02 ^a	0.085 ± 0.04 ^a	0.120 ± 0.02 ^a	0.111 ± 0.02 ^a
Neohesperidin dihydrochalcone	0.010 ± 0.001 ^a	0.010 ± 0.002 ^a	0.011 ± 0.002 ^a	0.011 ± 0.001 ^a
Quercetin	0.065 ± 0.009 ^a	0.045 ± 0.05 ^a	0.012 ± 0.01 ^a	0.035 ± 0.03 ^a
(-)-Catechin	105 ± 10.6 ^a	173 ± 21.4 ^a	164 ± 67.6 ^a	152 ± 37.8 ^a
Rutin	1.38 ± 0.67 ^a	1.19 ± 1.31 ^a	0.597 ± 0.12 ^a	0.640 ± 0.16 ^a
Chrysin	0.089 ± 0.06 ^a	0.083 ± 0.06 ^a	0.100 ± 0.02 ^a	0.139 ± 0.09 ^a
Phlorizin	139 ± 14.2 ^{ab}	111 ± 9.13 ^b	157 ± 30.2 ^{ab}	182 ± 29.2 ^a
Galangin	0.085 ± 0.06 ^a	0.071 ± 0.05 ^a	0.105 ± 0.02 ^a	0.127 ± 0.09 ^a
Apigenin	0.019 ± 0.003 ^a	0.008 ± 0.004 ^b	0.006 ± 0.001 ^b	0.014 ± 0.005 ^{ab}
Avicularin	14.9 ± 1.02 ^a	15.5 ± 15.4 ^a	9.63 ± 2.52 ^a	13.2 ± 1.02 ^a
Cynaroside	0.928 ± 0.19 ^a	0.561 ± 0.14 ^a	0.721 ± 1.45 ^a	0.905 ± 0.32 ^a
Calycosin-7-O-β-D-glucoside	12.8 ± 6.21 ^a	6.88 ± 4.07 ^a	8.22 ± 4.06 ^a	7.088 ± 0.13 ^a
Naringin Dihydrochalcone	0.025 ± 0.002 ^a	0.025 ± 0.002 ^a	0.023 ± 0.003 ^a	0.027 ± 0.006 ^a
Afzelechin	0.085 ± 0.034 ^a	0.085 ± 0.034 ^a	0.014 ± 0.001 ^a	0.031 ± 0.009 ^a
Hesperidin	0.024 ± 0.002 ^a	0.024 ± 0.002 ^c	0.002 ± 0.002 ^b	0.013 ± 0.004 ^c
Taxifolin	0.077 ± 0.007 ^a	0.077 ± 0.007 ^a	0.063 ± 0.012 ^a	0.057 ± 0.015 ^a
Engletin	0.013 ± 0.003 ^a	0.013 ± 0.003 ^a	0.006 ± 0.001 ^a	0.012 ± 0.009 ^a
Oroxin A	0.04 ± 0.006 ^a	0.04 ± 0.006 ^c	0.009 ± 0.001 ^{bc}	0.019 ± 0.001 ^{ab}
Baicalin	0.012 ± 0.005 ^a	0.012 ± 0.005 ^a	0.012 ± 0.004 ^a	0.009 ± 0.004 ^a
Kaempferol	0.05 ± 0.015 ^a	0.05 ± 0.015 ^b	0.021 ± 0.001 ^b	0.023 ± 0.008 ^{ab}
Hyperoside	9.14 ± 1.42 ^a	9.14 ± 1.42 ^a	9.09 ± 0.01 ^a	6.49 ± 2.47 ^a
Isorhamnetin	0.014 ± 0.001 ^a	0.014 ± 0.001 ^a	0.014 ± 0.001 ^a	0.013 ± 0.001 ^a

Values with different letters in the same line (a–c) are significantly different ($p < 0.05$) from each other. Values are given as the mean ± standard deviation.

From Figure 2A, it can be seen that the major identified metabolites in apple fruits treated with different methods include chalcones, flavanones, flavanonols, flavanones, flavones, flavonols, flavanols, and isoflavanones. Among these metabolites, followed by flavones (28%), chalcones (10%), flavanols (10%), flavanones (8%), flavanonols (8%), flavanones (3%), and isoflavanones (3%), flavonols (31%) present the largest proportion. It can be seen that flavonols and flavones are two major metabolites in apples. This finding is consistent with the study results of Chen et al. [14].

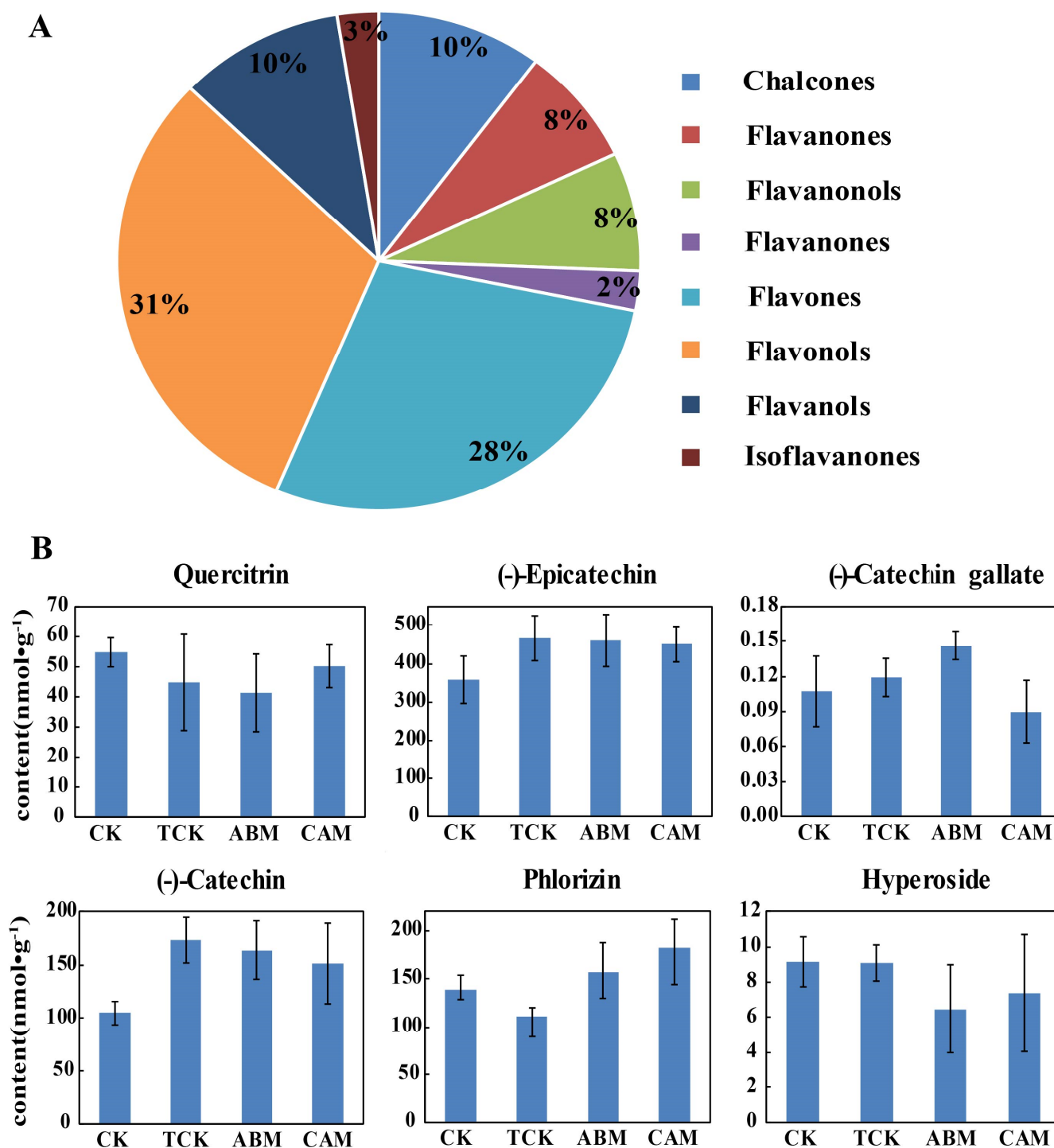


Figure 2. Classification results of flavonoid metabolites (A); Analysis results of important flavonoid metabolites in fruits (B).

Figure 2B presents the analysis results of important flavonoid metabolites in apple fruits. The results indicate that there are not great variations in the contents of Quercitrin, (-)-Epicatechin, (-)-Catechin gallate, (-)-Catechin, and Hyperoside between treatment groups and control groups, with no uniform interaction pattern among these groups. Apples in the treatment groups present more significant influences on the content of Phlorizin than those apples in the control groups, with less significant effects on the content of Hyperoside presented by apples in the treatment groups than those effects presented by apples in the control groups. Among those six important metabolites, only

Phlorizin presents lower contents in apples in the bagging treatment group than those contents in apples in the non-bagging groups, indicating that the syntheses of most of these metabolites do not rely on sunlight and are not affected by the spraying of non-bagging film agents.

4. Discussion

At present, there are few research reports on the effects of spraying non-bagging film agents on the quality and mineral elements of fruits. Under such a circumstance, this paper has systematically investigated the influences of spraying non-bagging film agents on the physical and chemical indexes and the contents of mineral elements and flavonoid metabolites of apple fruits. The study results show that spraying a non-bagging film agent is conducive to the accumulation of mineral elements in fruits and has a relatively small effect on the contents of their flavonoid metabolites, thus being feasible to be used in the non-bagging cultivation of apples, and these results are consistent with the study results of Cheng [15] et al.

In this study, compared with apples in the control groups, apples in the non-bagging film agent spraying treatment groups all present significantly increased contents of titratable acid, with no significant differences among their hardness and contents of soluble solids. This result is similar to the previous study results of other scholars, with some minor differences. One possible reason is that the effects of those bio-protective films applied in this study have coincided with the development and metabolic time of fruits, and another possible reason lies in the fact that these effects are related to the major components and concentrations of those bio-protective films applied.

Apples contain a great deal of elements such as magnesium (Mg), calcium (Ca), iron (Fe), and others that are beneficial to the human body [4]. Mineral element content plays an important role in judging fruit quality and in maintaining the normal physiological activities of the human body [16,17]. It is well known that phosphorus (P), potassium (K), calcium (Ca), and trace element iron (Fe) are essential for fruit quality [18]. In recent years, many studies have explored the effects of mineral nutrients on plant growth, fruit development, quality, and preservation of fruit, and their molecular mechanisms, especially the effects of P and K on fruit quality. The results of this research indicate that the contents of most elements of apples in the non-bagging film agent treatment groups are all higher than those contents of apples in the control groups, with increased total amounts of mineral elements. Therefore, this study has provided a new idea for the investigation of maximizing the nutritional value of apples and a reference for the further popularization and application of non-bagging cultivation. At the same time, this study has provided basic data support for the in-depth exploration of the molecular construction mechanisms of mineral elements in plant cell structures and the interactions and functions of these mineral elements.

Vitamin C, organic acid, sugar, flavonoids, and phenols are plant metabolites whose contents are regulated by the plant genetic and environmental factors. Some research has shown that humic acid substances, as an environmental factor, can affect plant metabolism by regulating the plant protease activity or inducing protease synthesis [19]. Polyphenols are a large group of bioactive plant compounds (over 8000) and have two main classes called non-flavonoids and flavonoids [20]. The non-flavonoids compounds include phenolic acids, stilbenes, and lignans, and the flavonoids compounds include flavonols, anthocyanidins, anthocyanins, isoflavones, flavones, flavanols (or catechins), flavanones, and flavanonols [21]. The main polyphenols found in apples are anthocyanins, dihydrochalcones, flavanols, flavonols, hydroxybenzoic acid, and hydroxycinnamic acid that play an important role in the healthful properties of apples [22]. The major flavonoid metabolites contained in apples include dihydrochalcones, flavanols, and flavonols [13,23], which is consistent with the results obtained in this study. The influences of different treatment methods on the contents of 39 metabolite types in apples were analyzed in this study, with the results showing that only Astragalin, Tiliroside, Homoplantagin, Phlorizin, Apigenin, Hesperidin, Oroxin A, and Kaempferol present significant differences in their contents,

with insignificant differences among the contents of all other metabolites, indicating slight influences of non-bagging film agents on the contents of flavonoid metabolites in apples. Some literature has reported that the composition and antioxidant capacities of phenolic compounds will be affected by the bagging treatment of fruits [24], with significantly reduced contents of flavonoids in fruit pulp [25], which is similar to, but slightly different from, the results obtained in this study.

As a new material, non-bagging film agent has the prospect of replacing fruit bagging in the application and popularization of non-bagging cultivation of apples. However, because the non-bagging cultivation of apples has high requirements on tree vigor, orchard management level, and complementary measures in non-bagging cultivation, it is still necessary to carry out further in-depth research to clarify the complementary measures for this cultivation technique.

There are some limitations in this study. With the bagging of apples, the contact between fruits and pesticides is reduced, thus effectively reducing pesticide residues on apple fruits. Therefore, it is necessary to deeply explore the components of non-bagging film agents and monitor the pesticide residues of fruits sprayed with non-bagging film agents to evaluate their safety.

5. Conclusions

Most apples in China have been bagged during cultivating to prevent pests and diseases in earlier stages and promote coloration and mitigate fruit russetting in later stages. In this study, a comparative analysis has been performed on the bagging and non-bagging treatment methods of apples, with the conclusion that the total contents of mineral elements in apples can be significantly increased with the spraying of two kinds of non-bagging film agents, with relatively small effects on the contents of flavonoid metabolites in apples. Therefore, it is suitable to use these two non-bagging film agents in the non-bagging cultivation of Fuji apples.

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