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Evaluation of Zinc Concentrations in Fruit from Various Pear Strains and Cultivars in China for Establishing a Standard for Zinc-Enriched Pears

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Abstract: Zinc (Zn)-enriched pears, which are derived from genetically related cultivars or crops fortified using agronomic methods, have the potential to partly satisfy the human demand for Zn nutrition and diversify consumer choices. However, a standard for the Zn fortification level in pears is lacking, and the disparity in literature-reported fruit Zn concentrations can vary by substantial amounts. Before investigating the Zn concentrations in fruits of the main pear cultivars in China, common sample preparation methods were compared. Among the pre-treatment methods tested, the freeze-dry technique had a greater degree of discrete variation, whereas oven drying (fresh weight) was the optimal method for determining fruit Zn concentrations. Based on the optimal method, no significant distribution patterns of fruit Zn concentration were found among the regions and strains examined. The averaged pulp Zn concentration in all 26 cultivars was 0.72 mg kg^{-1}, with the Hongxiangsu, Jinfeng, and 420 cultivars having the highest concentrations. Combined with the findings from our previous field experiments on Zn-fortified pears, a Zn concentration of $\geq$0.90 mg kg^{-1} is the suggested standard for pear enrichment. These results help us to better understand pear Zn nutrition levels and facilitate the marketisation of the fortified fruit.

Keywords: pear; fruit Zn concentration; sample preparation; quality standard; Zn-enriched cultivar

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1. Introduction

Pears (*Pyrus* spp.), which are usually eaten fresh or used for the production of processed foods such as dried fruit, juice, fruit wines, purees, jellies, and jams, are universally embraced and enjoyed by people worldwide [1]. Zinc (Zn) is an essential nutrient for the normal growth and development of pear trees. A lack of Zn absorption leads to an imbalance in Zn homeostasis in pear trees, which affects the health of crops, causing a decline in crop yields and quality and destruction of the stability of the orchard production system. Pear trees deficient in Zn would require additional supplementation of the essential mineral to meet their physiological needs. Zn is also an indispensable nutrient for human health. However, insufficient Zn intake due to nutritional imbalance or hidden hunger is a global issue, with approximately two billion people facing health risks associated with Zn deficiency [2–4]. The prevalence of hidden Zn deficiency is particularly high in low- and middle-income countries in Africa, South Asia, Southeast Asia, and Latin America. Dietary diversification is the best way for individuals to solve Zn deficiency, with special attention paid to avoiding the consumption of refined foods for a long time and noting the nutrition of coarse and fine grains. Through a diversified diet, individuals can ensure the intake of various nutrients from different food sources to prevent nutritional imbalances caused by a monotonous diet and meet their diverse nutrient needs, including Zn. Animal-based foods, such as red meat, seafood, poultry, and eggs, are rich in Zn and serve as important sources for achieving adequate intake of the mineral. However, not everyone is able to diversify their diet or consistently access sufficient amounts of animal-based foods to achieve a well-rounded and balanced diet [5,6]. In fact, many developing countries rely heavily on plant-based foods as the primary source of nutrition.

However, Zn is generally found in limited concentrations in plant-based foods, including pears and pear-based products (<25 mg kg^{-1} fresh weight (FW)), compared with its levels in animal-based foods (usually >40 mg kg^{-1}). Therefore, in agricultural countries that mainly grow crops, children and pregnant women (who generally have higher Zn requirements) are more susceptible to Zn deficiency and face greater challenges in addressing this issue through dietary diversification [7]. Aside from determining whether there are pear cultivars that contain high Zn concentrations, we were keen to investigate if agricultural measures can be employed to enhance the Zn level in the fruit. Such fortified pears for human consumption could help to partly supplement Zn levels beyond what ordinary pear cultivars offer. If Zn fortification through agricultural manipulation is possible, this approach could also facilitate product differentiation in the pear market, enriching the fruit offerings and mitigating competition resulting from homogeneity. Although no Zn-enriched pear cultivars have been reported to date, researchers have already attempted to improve fruit Zn concentrations by directly spraying the mineral onto crops [8,9]. After conducting technical enhancement studies on different spraying methods, we discovered that the Zn concentration in pear fruits could be increased using a chelated Zn fertiliser at high spraying concentrations, which has been successfully verified across various pear varieties in China [10]. Previous research on Zn fortification of produce and subsequent marketisation technologies have been conducted and applied on crops such as corn [11,12], rice [13], wheat [14,15], and sweet potatoes [16]. However, compared with those crops, the further development of Zn-enriched pears faces two main challenges.

First, the absence of a standardised Zn concentration in Zn-enriched pears results in a lack of understanding about the characteristics of the fortified fruit. The standard of Zn-enriched pears serves as the foundation for promoting and selling the fruits while also playing a pivotal role in leading and driving the healthy development of the fortified-pear market. By contrast, the research and commercialisation of Zn-enriched rice are relatively well developed. The HarvestPlus project, an international initiative focussed on crop biofortification, aimed to enhance the Zn concentration of wheat grains from 25 mg kg^{-1} to 37 mg kg^{-1} and that of rice from 16 mg kg^{-1} to 28 mg kg^{-1} [17]. Consequently, a Zn concentration of 28 mg kg^{-1} can be considered the minimum threshold or technical realisation goal for the production of Zn-enriched rice. Currently, there are no national or industrial standards for Zn-enriched crops in China, and only local and enterprise standards for 'Zn-enriched rice' are available as references. For example, the Zn-rich rice enterprise standard (Q/JCMY 01-2018) formulated by Jincheng Rice Co., Ltd. (He County, Anhui Province, China) specifies a Zn concentration range of 35–45 mg kg^{-1}. To date, there is a lack of studies on the Zn concentration in specifically labelled 'Zn-rich' pears, let alone reports establishing a universally accepted and standardised Zn level in the fruit. Given that there are currently six pear strains and over 20 primary pear cultivars cultivated in various countries throughout the world, conducting a worldwide survey of the Zn levels in pear germplasm resources is essential for establishing recognised standards.

The second basic problem is the need to establish a more scientific method for determining the Zn concentration in pear fruit. Unlike gramineous crops, pear fruits remain large, watery, and sticky after drying. Although many research studies on the Zn concentration in pear fruit have been performed, there are no uniform and more-recognised methods for obtaining the measurements, resulting in a large variation in reported concentrations, which range from 0.01 to 1.00 mg kg^{-1} [8,10,18]. Although drying and freeze-drying are two commonly used pre-treatment methods, the difference between them in terms of the resulting moisture and Zn concentrations measured has never been verified. We assume that these pre-treatment methods would result in different measurements because of the difference in the degree of drying of the sample. Overall, a scientifically verified sample preparation method is a prerequisite for the standardised determination of pear Zn concentrations and is fundamentally essential for analysing the aforementioned problems.

The specific objectives of the present study were to determine (1) the optimal sample pre-treatment method for measuring fruit Zn concentrations and (2) the Zn concentration at which pear fruits can be labelled as 'Zn enriched'. Owing to limited funds and time, we focussed solely on collecting pear tree varieties from China. The diverse climate types and soil conditions in China, coupled with the extensive range of available pear varieties, enabled our collection to reasonably represent the global Zn concentration levels found in pear fruits to a certain extent. Aside from enhancing our knowledge about the Zn nutrition level in fruits, our research results could also play a notable role in promoting the marketisation of Zn-enriched pears.

2. Materials and Methods

2.1. Investigation on Fruit Zn Concentration of Pear Germplasm Resources

Pear fruits of 26 cultivars were predominately collected from the National Pear Germplasm Resources Nursery in Xingcheng, Huludao City, Liaoning Province, China, and the Pear Branch Center (Zhengzhou) of the National Horticulture Germplasm Center in Zhengzhou City, Henan Province, China (Figure 1). The growth areas of the collected pear cultivars are distributed over ten provinces, which contributed three quarters of the Chinese domestic pear production in recent years (SSB, 2022). Three representative adult fruit trees were selected for each pear cultivar, and at commercial maturity, ten representative fruits of uniform size, with no mechanical damage and no harm from diseases and insects, were randomly picked from the middle periphery of the crown. All fruits were weighed to calculate the average single fruit fresh weight. Per tree, three fruits with a close-to-average weight were selected to determine the Zn concentration. All pear fruits were peeled and the Zn concentrations in the pulp were determined as described below. The average Zn concentrations for the entire country, northern China, southern China, and per province, pear strain, and cultivar were calculated.

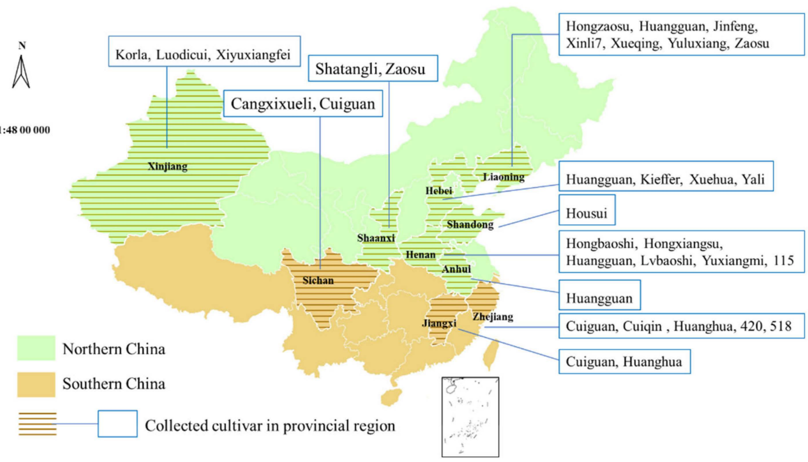

Figure 1. Sample collection. Twenty-six pear cultivars locations distributed in ten provinces of China. Pear cultivars indicated in green font were used in Zn fertilization efficiency tests. **Note:** Kieffer is a

European pear cultivar (*Pyrus communis* L.); Cangxixueli, Cuiguan, Cuiqin, Housui, Huanghua, Lvbaoshi, Xueqing, and Yuxiangmi are sand pear cultivars (*Pyrus pyrifolia* (Burm. f.) Nakai); Hongzaosu, Huangguan, Xuehua, Yali, and Zaosu are white pear cultivars (*Pyrus Bretschneideri* Rehd.); three cultivars in Xinjiang, and Yuluxiang are Xinjiang pear cultivars (*Pyrus sinkiangensis*); Shatangli is an Ussurian pear variety (*Pyrus ussuriensis* Maxim.); Hongbaoshi, Hongxiangsu, Jinfeng, Xinli7, and Yuluxiang are newly bred hybrid cultivars that are difficult to categorize; 115, 420, and 518 are unregistered cultivars that do not have officially registered names.

2.2. Pre-Processing of Pulp Samples

All fresh fruits collected were cleaned in clean water and distilled water in order, and then partitioned into pericarp (2 mm thickness), core (25 mm diameter), and ring-shaped pulp parts (5 mm thickness) using a stainless peeler with corer (Figure 2). As the pulp samples readily started browning, they were treated as quickly as possible to avoid environmental effects. The slicer (Figure 2d–f) could divide the ring-shaped pear into 8 parts, and 4 equal samples were collected by diagonal method. The trapezoid-shaped pieces can ensure that the sample of 4 equal parts is more uniform than that of traditional methods (Figure 2a–c). Trapezoid-shaped pulp samples of approximately 10.0 g were prepared to compare three methods for determining the Zn concentration in fresh fruits.

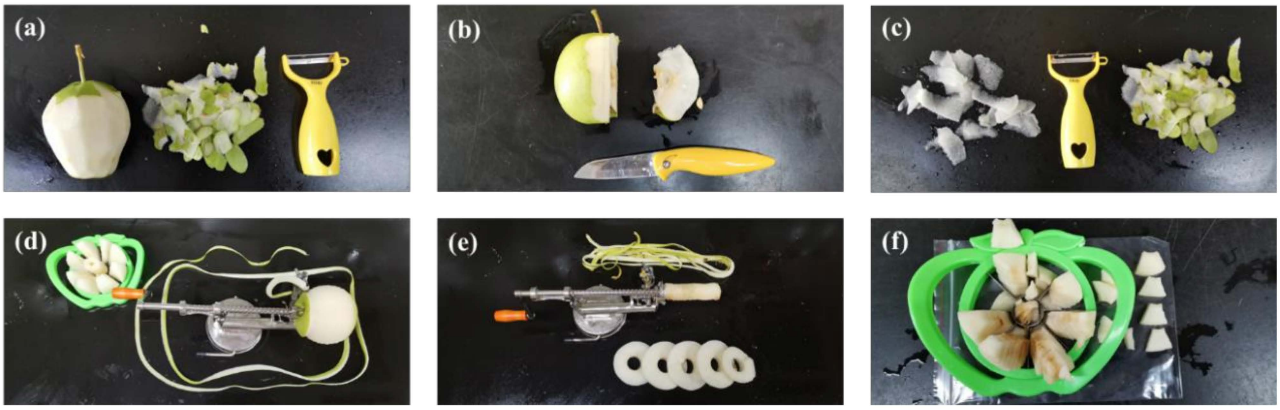

Figure 2. Specimen preparation of pear fruit. (**a**) Common Peeler; (**b**) common knife used to cut the peeled fruit into slices; (**c**) common peeler used to cut peeled fruit into pieces; (**d**) stainless peeler with corer used in the study; (**e**) a pear fruit divided into peel, core, and ring-shaped pulp pieces generated using the stainless peeler with corer; (**f**) stainless slicer used to slice the ring-shaped pulp mass into trapezoid-shaped pieces in the study.

2.3. Determine Method of Fruit Zn Concentration

One fresh sample was directly used to determine the Zn concentration according to the procedure used for non-pulp samples (termed 'wet' method). One fresh sample was frozen at –80 °C and then dried in a freeze-dryer at –40 °C (SCIENTZ-10N; Ningbo Scientz Biotechnology, Ningbo, China). The dried sample was weighed and the fruit moisture concentration was calculated. Then, the dried sample was cut into pieces using ceramic scissors, and 0.5 g was used to determine the Zn concentration (termed 'freeze-dry' method). The two remaining fresh samples were dried to a constant weight at 105 °C for the first 15 min and then at 65 °C, and their moisture concentrations were calculated. One of the dried samples was cut into pieces using ceramic scissors, and 0.5 g was used to determine the Zn concentration (termed 'oven-dry' method). All tested samples were digested with mixed acid liquid of HNO_3-$HClO_4$-H_2O_2 (5:2:1, v v^{-1}, GR) in a polytetrafluoroethylene tube, and then were determined by ICP-AES (Thermo 6300, Thermo Fisher Scientific, Waltham, MA, USA). Quality controls were carried out with Analysis Standard Material (GB[E] 080684) and blank samples.

The fruit Zn concentration of pear based on fresh weight and dry weight could be calculated as follows:

$$C_{FW} = C_{DW} \times (1 - W) \quad (1)$$

where, C_{FW} and C_{DW} represent the Zn concentration (mg kg^{-1}) in the fresh fruit and dried fruit, respectively; W represents the fruit water content (%); and *FW* and *DW* represents the fresh weight and dry weight of one same fruit sample, respectively.

2.4. Statistical Analysis

Data were analysed using the software of STATISTICA v. 5.5 (TIBCO Software, Palo Alto, CA, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). Tables and charts were also made by Microsoft Excel 2016. Data are expressed as the mean value ± standard deviation (SD). The Shapiro–Wilk test was used to assess variable normality among treatments. Means were compared by one-way analysis of variance followed by Tukey's honestly significant difference test. The significance level using LSD minimum significant difference method was set at $p < 0.05$.

3. Results

3.1. Determination of the Optimal Pre-Treatment Method for Obtaining Pear Zn Concentrations

Three commonly used sample pre-treatment methods were compared, because there is currently no standard method used (Figure 3). The fruit Zn concentrations in samples pre-treated with the oven-dry method (DW) were higher than those in samples prepared using the freeze-dry method (DW), owing to the difference in the moisture content (Figure 4). The moisture content of the fresh fruits was between 80% and 95% and increased with increasing fruit weight. The moisture content of the oven-dried (DW) samples was higher than that of the freeze-dried samples (DW), owing to the more thorough drying achieved by the freeze-dry method. The averaged moisture content of the samples pre-treated with the oven-dry method (DW) was 91.0%, which was 4.3 percentage points higher than the value obtained for the freeze-dried samples (DW). The Zn concentrations based on FW were similar among the fruit samples, regardless of the pre-treatment method used. Because there was a greater degree of discrete variation for the freeze-dry method (DW), the oven-dry method (FW), which is the most widely used pre-treatment technique, was chosen for the subsequent determination of fruit Zn concentrations.

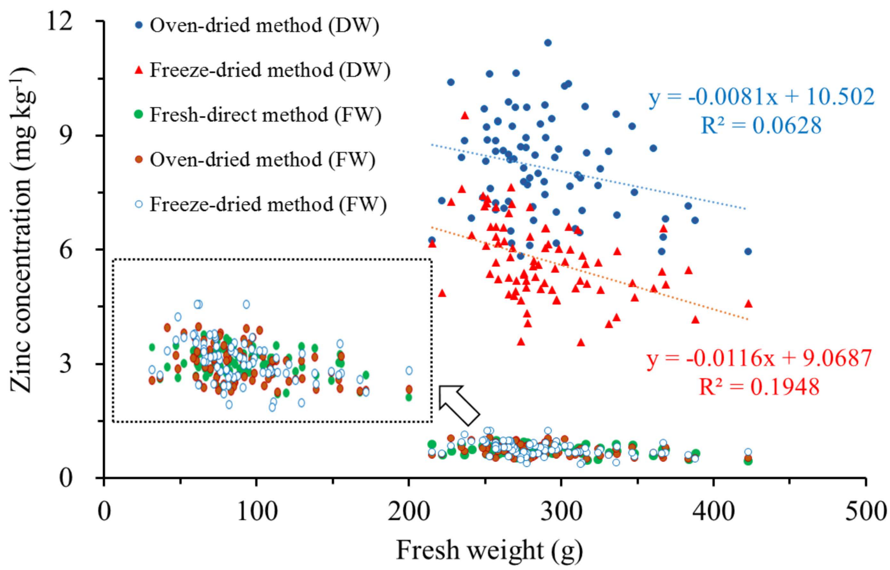

Figure 3. Comparison of determination methods for Zn concentration in fruit of Cuiguan cultivar. **Note:** DW—dry weight; FW—fresh weight.

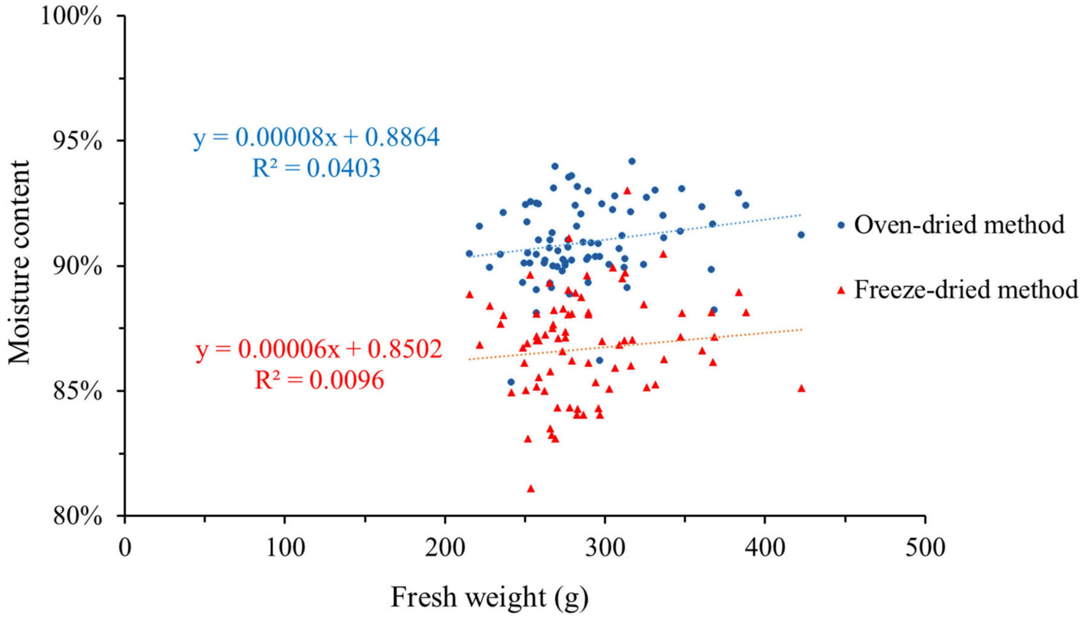

Figure 4. Moisture content of fresh fruits measured using the 'oven-dry' and 'freeze-dry' methods.

The distribution frequencies of fruit Zn concentrations for the three pre-treatment methods are shown in Figure 5. The overall Zn concentrations from all three methods ranged from 0.25 to 1.24 mg kg^{-1}. The distribution frequency for the 0.25–0.49 and 1.00–1.24 mg kg^{-1} Zn concentration ranges was less than 10%, respectively, whereas it was more than 80% at Zn concentrations between 0.50 and 0.99 mg kg^{-1}. Additionally, the distribution frequency showed that the degree of discrete variation for the freeze-dry method (DW) was greater than that for the other two methods.

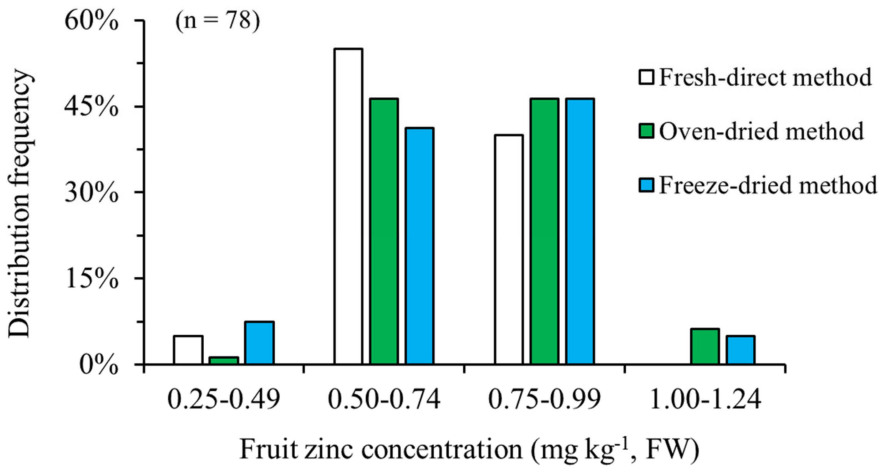

Figure 5. Zinc concentration distribution frequencies for three determination methods used. **Note:** FW—fresh weight.

3.2. Comparison of Zn Concentrations in Pear Fruits at the Regional, Strain, and Cultivar Levels

The Zn concentrations at the regional levels is shown in Figure 6. The average Zn concentration in all fresh fruits collected was 0.72 mg kg^{-1}, and the values did not differ significantly ($p < 0.05$) between fruits from northern and southern China, and no differences were found among provinces.

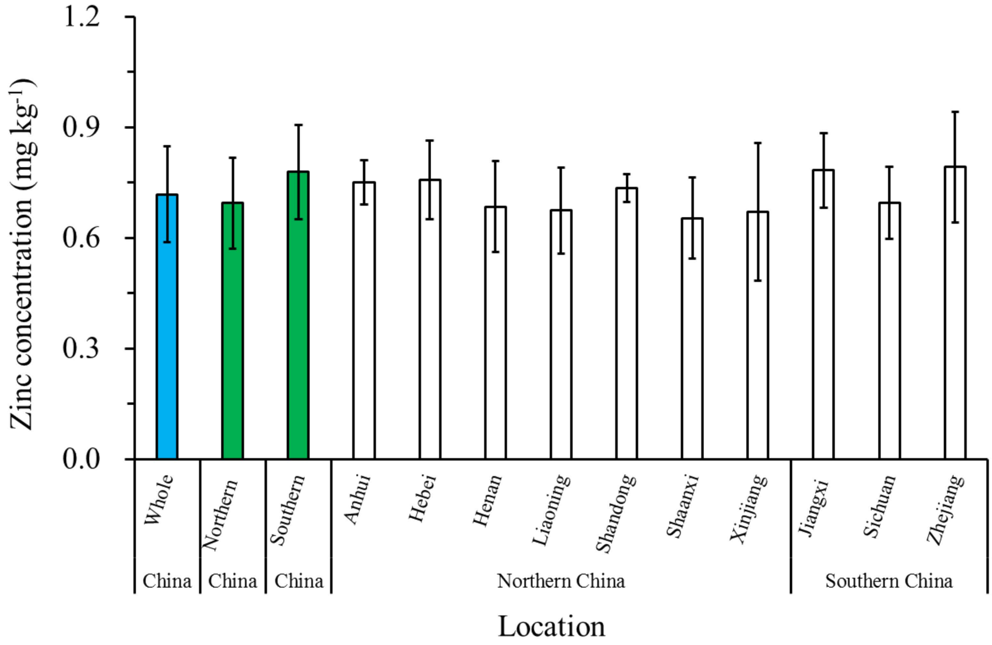

Figure 6. Comparison of the Zn concentrations in pear strains from different regions across China. **Note:** Data are expressed as the mean ± standard deviation. The distribution of North and South China can be seen in Figure 2.

The Zn concentrations in the different pear strains were in the following order: European pear > white pear > Xinjiang pear > hybrid pear > Ussurian pear > sand pear (Figure 7). European pears had the highest Zn concentration, which reached 0.87 mg kg^{-1}. Among the 26 pear cultivars evaluated, the Zn concentration ranged from 0.49 to 0.92 mg kg^{-1} (Figure 8). Hongxiangsu, Jinfeng, and 420, which are newly cultivated varieties, were the only cultivars with a Zn concentration higher than 0.80 mg kg^{-1}. The lowest Zn concentrations (<0.55 mg kg^{-1}) were found in the Hongzaosu, Xinli7, and Xiyuxiangfei cultivars.

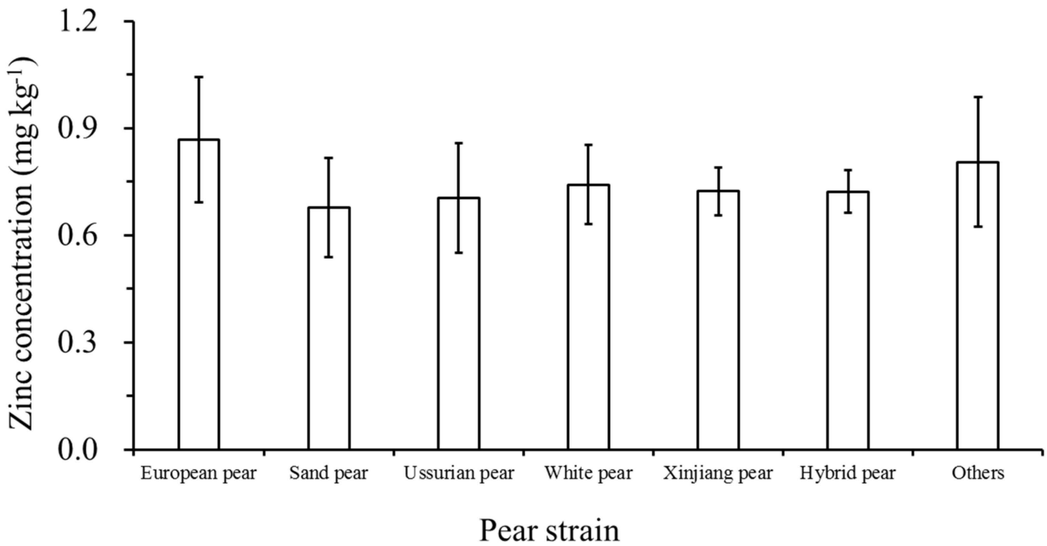

Figure 7. Distribution of Zn concentration in different pear strains. **Note:** Data are expressed as the mean ± standard deviation.

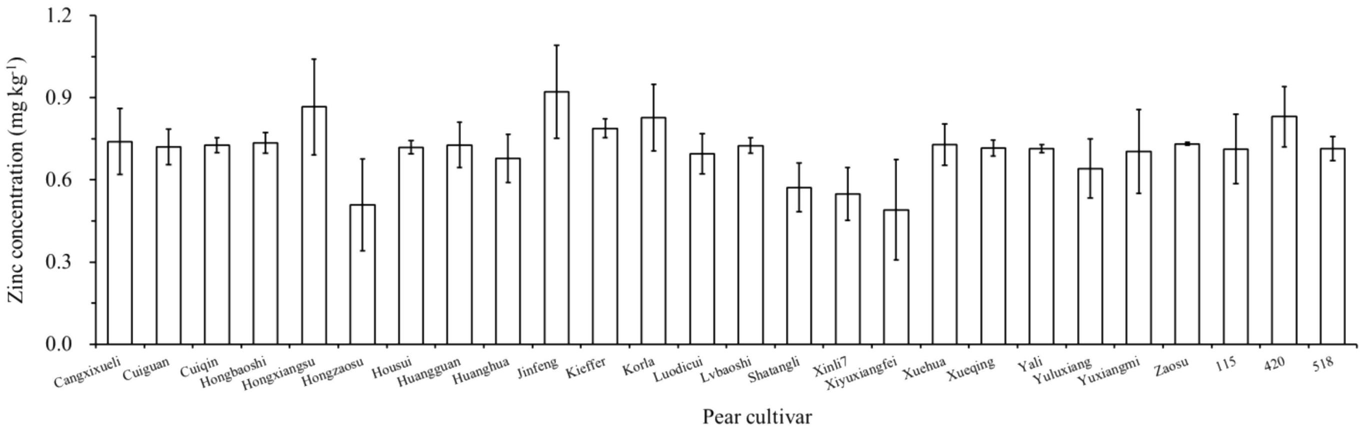

Figure 8. Distribution of Zn concentration in different pear cultivars. **Note:** Data are expressed as the mean ± standard deviation. Kieffer is a European pear cultivar (*Pyrus communis* L.); Cangxixueli, Cuiguan, Cuiqin, Housui, Huanghua, Lvbaoshi, Xueqing, and Yuxiangmi are sand pear cultivars (*Pyrus pyrifolia* (Burm. f.) Nakai); Hongzaosu, Huangguan, Xuehua, Yali, and Zaosu are white pear cultivars (*Pyrus Bretschneideri* Rehd.); Korla, Luodicui, Xiyuxiangfei, and Yuluxiang are Xinjiang pear cultivars (*Pyrus sinkiangensis*); Shatangli is an Ussurian pear variety (*Pyrus ussuriensis* Maxim.); Hongbaoshi, Hongxiangsu, Jinfeng, Xinli7, and Yuluxiang are newly bred hybrid cultivars that are difficult to categorize; and 115, 420, and 518 are unregistered cultivars that do not have officially registered names.

4. Discussion

4.1. Choice of Method for Sample Preparation

Various sample pre-treatment methods are used when determining fruit Zn concentrations, including peeling, coring, sectioning, cleaning, drying, and digestion [19,20]. In our study, the digestion solution easily overflowed during the digestion of fresh and freeze-dried pear samples, and a long pre-digestion period at low temperatures was required. Owing to the differences in moisture content of the pear samples, the comparability of Zn concentrations based on fruit DW was poor, and we determined that it was best to use measurements based on FW instead. The moisture content is an essential indicator of fruit quality, and the drying process is essential for determining the fruit moisture level. For these reasons, we regarded oven drying (FW) as being optimal among the five methods tested for determining the fruit Zn concentration. Many studies have shown that different processing techniques have a significant influence on the concentration and form of Zn. In the future, the Zn concentrations of pear products, such as pear juice and pear dried fruit, should be compared in different ways to avoid excessive Zn loss [20].

4.2. Dilution Effect of Fruit Zn Concentration

Zn-enriched pear fruits have rarely been reported, not only because of the variability in results but also because of the lack of a widely recognised standard. Many studies have reported that soil Zn application can increase the mineral concentration in fruits. Although we did not measure the Zn concentration in soil, it is known that the Zn levels of soils in northern and southern China differ greatly, and we found that the concentrations in the fresh fruits were generally not noticeably different. This indicates that pear fruits can maintain a stable Zn state without causing physiological problems. The Zn concentration in pears varies according to the location of the fruit on the tree, moisture content, and fruit maturity. Sampling locations, such as whether the fruits were south- or north-facing or located at the crown or bottom of the tree, did not noticeably affect the Zn concentration. Furthermore, the phenomenon of the Zn concentration or dilution effect cannot be ignored. In recent decades, the pursuit of high-yielding breeds has led to Zn concentrations being generally lower in the high-yield crop varieties than in earlier low-yield cultivars, indicating the existence of a concentration effect [5]. The flesh of low-weight pears was found to have a higher Zn concentration than that of high-weight pears, and spraying with 1.6% NaCl markedly reduced the fruit weight of individual pears and increased the Zn concentration in the flesh [10,21]. This was also true for crops such as rice [13] and waxy maize [12], indicating that the Zn concentration effect in the edible parts of crops is universal. Therefore, heavier fruits should be preferentially selected for measuring Zn fortification in pears. This also indicates that the premise of Zn biofortification in the edible parts of crops is not to cause crop yield reduction, because the increase in Zn concentration in reduced-yield crops is partly due to the Zn concentration effect in the edible parts.

4.3. Standard Fruit Zn Concentration Level for Zn-Enriched Pears

Another problem with pear enrichment is the Zn attainment rate. Foliar spray applications of 0.1%–0.5% $ZnSO_4$ have been used for the Zn fortification of fruits [8,9]. However, fruit producers prefer not to use $ZnSO_4$, as it does not induce a sufficient level of Zn fortification and the overall attainment rate is too low for large-scale implementation. In our previous study, spraying 0.4% $ZnSO_4$ increased the fruit Zn concentration to 0.9 mg kg^{-1} [10]. However, if this were the standard, most cultivars would not meet the 60% attainment rate. By contrast, spraying with 1.5% ZnEDTA achieved a 100% mineral attainment rate for all cultivars. Moreover, 1.5% ZnEDTA induced Zn fortification with an attainment rate of more than 89%, if based on the standard for Zn-fortified pears ($\geq$1.00 mg kg^{-1}, FW). As an alternative, the mineral concentration could be further enhanced by spraying a Zn fertiliser onto the Zn-rich pear varieties identified in this study. We believe that the application of 1.5% ZnEDTA is the best method for improving the pear Zn concentration [10]. From the point of view of what can presently be achieved, a Zn concentration of $\geq$0.90 mg kg^{-1} (FW) is suggested as the standard for pears to be labelled as 'Zn enriched'.

Unexpectedly, we discovered several pear cultivars with higher Zn contents than the other varieties studied. A long breeding cycle is needed to attain Zn-enriched varieties of high quality and yield and with stress resistance. Genetic engineering can accelerate the mastering of genetic laws for attaining Zn enrichment traits, and modern breeding techniques can be used to purposefully combine related genes of Zn-rich varieties to accelerate the breeding process. Although transgenic technology has been successfully applied to certain grain crops, such as corn and wheat, its application to pears and other crops with complex genetic backgrounds lags in comparison. The combination of agronomic practices with transgenic technology is expected to expedite the process for breeding Zn-rich pear varieties [22,23]. In the future, the Zn concentration of pears should be investigated on a global scale, and technical and scientific efforts should be made to promote the marketisation of Zn-enriched pear fruit.

5. Conclusions

Among the sample preparation methods tested, oven drying (FW) was chosen as the most applicable for determining fruit Zn concentrations. Combined with the findings from our previous field experiment on Zn-fortified fruit, a Zn concentration of $\geq$0.90 mg kg^{-1} (FW) is suggested as the standard for labelling pears as 'Zn enriched'. Additionally, we identified three cultivars (Hongxiangshu, Jinfeng, and 420) with the highest fruit Zn concentrations amongst the varieties tested, and they should be further studied for the future development of Zn-enriched pears. The findings from our investigation of pear germplasm resources and comparison of pre-treatment methods for determining fruit Zn concentrations should help facilitate the marketisation of Zn-enriched pears.

Author Contributions: M.L.: data curation, methodology, software, writing—original draft preparation, visualization visualisation; H.Y.: data curation, resources, investigation; L.L.: data curation; Z.M.: investigation; J.Z.: investigation; S.T.: data curation; L.W.: funding acquisition, supervision, project administration; G.F., writing—review and editing; Y.L.: investigation, writing—review and editing; M.X.: data curation, writing—review and editing, validation. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

DW-dry weight; FW-fresh weight: GR-Guaranteed reagent.

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