Current Research in Food Science 5 (2022) 34–40

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

A quantitative detection of mung bean in chestnut paste using duplex digital PCR

Yingjie Liang, Dongwei Gao, Jie Dong, Lijun Guan, Zhiyong Li, Jin Liu *

Guangzhou Customs Technology Center, No. 66 Huacheng Avenue, Tianhe District, Guangzhou, China

ARTICLE INFO

Keywords:
Chestnut paste
Mung bean
Digital PCR
Quantification

ABSTRACT

Highly manufacturing process of chestnut paste leaves a considerable space for Economically Motivated Adulteration (EMA) with cheaper ingredients such as mung bean. In this paper a novel quantitative detection of mung bean in chestnut paste using duplex digital PCR was reported. Two sets of primers and probes were designed according to mung bean and chestnut specific genomic genes suitable for duplex droplet digital PCR (ddPCR) and duplex chip digital PCR (cdPCR) to set up a mass ratio quantitative detection method for mung bean, a common alternative plant-derived ingredient in chestnut paste products. The manufacturing process of chestnut paste products was considered to establish the linear relationship formula between mass ratio and gene copy number (CN) ratio of the two ingredients. The limits of quantification for gene CN concentrations (LOQ_{copy}) of mung bean and chestnut were both 6 copies/μL, at the same time a mass ratio of mung bean in chestnut paste range from 5% to 80% was able to be quantified accurately to provide technical support for the identification of fraudulent substitution or adventitious contamination.

1. Introduction

Many prepackaged foods are difficult to be identified real or fraud because of their highly manufacturing process such as grinding, high temperature and high pressure treatment, as well as the use of various food additives. It creates considerable conditions for Economically Motivated Adulteration (EMA), a serious food safety crime to use cheap ingredients to pass off as expensive ones, which could do great harm to food safety and fair trade (Everstine et al., 2013). EU horse meat scandal in 2013 attracted the eyeball of the whole world then accelerated the introduction of relevant supervisory inspection policies and technical detection measures on food adulteration of animal derived materials (Hsieh and Ofori, 2014). Actually food adulteration of plant derived materials is also widespread globally, passing cheap plant materials with similar taste and color off as expensive ingredients with the help of flavoring substances, especially in prepackaged paste products. For example, mung bean and chestnut flavoring are used to produce chestnut paste, kidney bean and lotus seed flavoring are used to produce lotus seed paste, as well as white gourd and durian flavoring are used to produce durian paste (Sun and Huang, 2017).

Chestnut is not only a traditional food in Europe and Asia for thousands of years, but also loved extensively by consumers in other continents. Food and Agriculture Organization of the United Nations (FAO) statistics indicated that there are only 2 commercially cultivated species planted for nut production worldwide, among which Chinese chestnut (*Castanea mollissima*) accounts for more than 86% of global amount with a production around 1.88 million tons, and European sweet chestnut (*Castanea sativa*) contributes for about 6% with a yield of 0.13 million tons (Baer et al., 2018). Chestnut paste is one of the most common high processed chestnut types to be massively used in pastries and desserts (Blaiotta et al., 2012). Adulteration with other materials in chestnut paste production occurs in order to reduce production costs and make higher profits. Mung bean is most commonly used to replace partial chestnut. Sometimes the situation is worse when a chestnut paste product is manufactured without any chestnut ingredient but only by mung bean and flavorings. After a series of manufacturing processes including cooking, grinding, flavor blending, shoveling and so forth, it is difficult to know the exact chestnut content by visual inspection or chemical/physical tests (Li et al., 2016).

In China, a national standard entitled “GB/T 21270-2007 Food Fillings” stipulates the content of chestnut ingredient no less than 60% (w/w) in chestnut paste excluding oil and sugar. A trade standard

* Corresponding author.
E-mail addresses: 441860140@qq.com (Y. Liang), margree@126.com (D. Gao), dongjie@gdciq.gov.cn (J. Dong), 1093749557@qq.com (L. Guan), lizy@iqtc.cn (Z. Li), ivfhonline@126.com (J. Liu).

https://doi.org/10.1016/j.crfs.2021.12.003
Received 21 June 2021; Received in revised form 10 December 2021; Accepted 12 December 2021
Available online 18 December 2021

entitled "SN/T 4419.9–2016 Food allergen detection with LAMP methods for export - Part9: Chestnut" and a provincial standard entitled "DB12/T 843–2018 Mung bean-derived ingredients detection - Qualitative PCR method" were implemented, providing limited qualitative technical measures to answer the questions of yes or no on the existence of chestnut and mung bean components in chestnut paste products. It is unable to give accurate and reliable evidence on the authentication of intended adulteration from accidental contamination caused by shared containers, facilities and manufacturing lines for these standards.

Although the necessity of establishment and application on EMA identification technique is so urgent and clearly seen, most of the researches (El Sheikha, 2019) focus on qualitative detection before digital PCR (dPCR) was involved in, which cannot answer the question of intended adulteration or accidental contamination.

dPCR has been demonstrated to be an efficient technique for the quantitative detection of target nucleic acids. According to Poisson Distribution, the statistical data for test results from separate PCR microchambers are corrected to calculate the exact CN concentration of the target nucleic acid in the sample (Baker, 2012; Quan et al., 2018). Compared with real time fluorescent PCR, dPCR has some obvious advantages. dPCR has no need for plotting a linear standard curve with gradient reference materials and accomplishes an accurate quantification on CN concentration of target nucleic acid sequence directly from an unknown sample (Whale et al., 2013). Therefore, there is no deviation caused by DNA extraction efficiency and amplification ability among unknown samples and reference materials. Besides, dPCR has a higher tolerance to PCR inhibitors inside sample substance since it depends on fluorescent signal counting at PCR end point rather than real time monitoring, thus has a lower demand on DNA purity of unknown samples. When it comes to processed foods, various PCR inhibitors inside complicated ingredients may cause significant deviation on amplification efficiency among samples and reference materials, which become one of the major origins of relevant quantification error (Dingle et al., 2013; Hindson et al., 2013).

Recently dPCR has been applied on different food detection areas such as transgenic component detection (Cottenet et al., 2019; Wan et al., 2016), meat adulteration identification (Floren et al., 2015) and plant-derived ingredient quantification (Scollo et al., 2016). Some of these dPCR methods have been introduced into national and industrial standards, such as "SN/T 5334.1–2020 Protocol of digital PCR for quantitatively detecting genetically modified plants and their derived products." and "GB/T 33526–2017 Genetically modified organism detection method by digital PCR".

Therefore, a quantitative detection method on the mass ratio of mung bean in chestnut paste based on the determination of DNA CN concentrations of mung bean and chestnut ingredients using duplex dPCR was reported in this work.

2. Materials and methods

2.1. Samples

Eighteen beans, nuts, cereal seeds and other plant materials commonly used as starchy ingredients in pastes (Table 1), as well as 13 chestnut pastes and relevant foods were purchased in local market or on domestic e-commerce platform, then were ground using IKA® Tube-Mill Control (IKA Works GmbH & Co., Germany).

2.2. DNA extraction

DNAs were extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) and DNA quantity was measured with NanoSpec spectrophotometer (Shimadzu, Japan). The volume of extraction reagents and liquid pipetting should be fixed to guarantee the accuracy of quantification to the most extent, including 700 μL Nuclei Lysis Solution, only 550 μL supernatant transferred after protein precipitation, and 50 μL DNA Rehydration Solution for DNA pellet dissolution. Besides, the 550 μL supernatant from protein precipitation was purified by mixing with 550 μL phenol-chloroform-isopentanol (25:24:1, volume ratio) and violent inversion for several times, only 400 μL supernatant from the centrifuged mixture after purification was transferred for isopropanol precipitation.

Table 1
18 plant materials commonly used as starchy ingredients in pastes.

No.	Common name	Scientific name
1	Chestnut	*Castanea mollissima*
2	Cone chestnut	*Castanea henryi*
3	Mung bean	*Vigna radiata*
4	Adzuki bean	*Vigna angularis*
5	Rice bean	*Vigna umbellate*
6	Soybean	*Glycine max*
7	Cowpea	*Vigna cylindrica*
8	Pouch bean	*Phaseolus coccineus*
9	Kidney bean	*Phaseolus vulgaris*
10	Lotus seed	*Nelumbo nucifera*
11	Hazelnut	*Corylus heterophylla*
12	Peanut	*Arachis hypogaea*
13	Gorgon fruit	*Euryale ferox*
14	Coix seed	*Semen Coicis*
15	Rice	*Oryza sativa*
16	Wheat	*Triticum aestivum*
17	Maize	*Zea mays*
18	Chinese yam	*Dioscoreaopposita*

2.3. Duplex dPCR

Two sets of primers and probes were designed according to mung bean and chestnut specific genomic DNA sequences using Primer Express 5.0 Software and synthesized by Shanghai ShineGene Molecular Biotechnology Co. LTD (Shanghai, China). The sequences and sources of primers and probes as well as the labeling were listed in Table 2.

ddPCR analyses were performed on a QX200™ Droplet Digital PCR System (Bio-Rad, USA) according to the supplier's recommendations. The 20-μL reaction cocktail for duplex ddPCR was composed of 10 μL ddPCR™ Supermix for Probes (no dUTP) (Bio-Rad Laboratories, USA), 0.4 μM primers, 0.2 μM probes, and 2 μL DNA templates. Reaction procedure was 5-min denatureation at 95 °C initially, then 49 cycles of 15-sec denaturation at 94 °C and 60-sec extension at 60 °C, and finally PCR product storing at 12 °C. Data analysis was performed with QuantaSoft™ Software V1.7.4.

cdPCR analyses were performed on a QuantStudio™ 3D Digital PCR System (Thermo Scientific, USA) according to the supplier's recommendations. The 15-μL reaction cocktail for duplex cdPCR was composed of 7.5 μL QuantStudio® 3D Digital PCR Master Mix v2 (Applied Biosystems by Life Technologies, USA), 0.4 μM primers, 0.2 μM probes, and 1.5 μL DNA templates. Reaction procedure was 10-min denatureation at 96 °C initially, then 49 cycles of 30-sec denaturation at 98 °C and 2-min extension at 60 °C, and finally PCR product storing at

Table 2
Primers and probes for duplex dPCR.

Primers and Probes	Sequences (5' - 3')	Amplicon length/bp	GenBank No.
Chestnut-F	AAGCCTAAAATGCGACACTACG	119	AH015527.2
Chestnut-R	TGTCTCCAAGCCCCAACG		
Chestnut-P	FAM-CCTCCACTGCCTTGACGAGGAAGC-BHQ1		
Mung bean-F	GACCGGCAGCTTATGCTTCA	80	XM_022781395.1
Mung bean-R	AACAGCGGCTAACTCGATGTC		
Mung bean-P	VIC- CAATTAAAGTCGCATGAGAG -MGB		

10 °C. QuantStudio™ chip analyzer and QuantStudio™ 3D Analysis Suite™ Cloud Software were used for data analysis.

2.4. Specificity analysis on primers and probes

To test the specificity of the presented dPCR methods, DNAs of 18 various kinds of plants listed in Table 1 were analyzed.

2.5. Evaluation on limit of quantification for DNA CN concentration (LOQ_{copy})

DNAs extracted from chestnut powder and mung bean powder with known DNA CN concentrations determined using duplex ddPCR were diluted with ddH_2O to100 copies/μL, 50 copies/μL, 20 copies/μL, 10 copies/μL, 5 copies/μL and 1 copies/μL. The diluted DNAs were then determined in triplicates using ddPCR and cdPCR to evaluate their LOQ_{copy}.

2.6. Correlation analysis on mass and DNA CN concentration

DNAs extracted in triplicates from chestnut powder and mung bean powder in a serial weight of 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg and 50 mg were determined using duplex ddPCR and cdPCR. A statistical analysis was performed to find out the correlation between powder mass and CN concentration by plotting mass-CN concentration curves.

2.7. Establishment of "mass ratio-DNA CN ratio" formulas

Two sets of spiked samples with a gross weight of 100 g per sample were prepared with a serial mass ratio of mung bean including 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70% and 80%. The weighted mung beans and chestnuts were mixed and blended into paste with 50 mL ddH_2O. For set 2, bean coats were dehulled by boiling the weighted mung beans in 10 g/L $NaHCO_3$ solution before mixing with the weighted chestnuts (Mubarak, 2005). DNAs extracted in triplicates from 2 sets of spiked samples in weight of 50 mg were determined using duplex ddPCR and cdPCR. "Mass ratio-DNA CN ratio" formulas were established by statistical analysis and curve plotting.

2.8. Validation on linear range of mass ratio quantification

Six simulated chestnut paste samples were prepared from mung beans and chestnuts with a gross weight of 100 g containing 5%, 10%, 20%, 40%, 60% and 80% mung beans. A universal manufacturing procedure was followed during the preparation. Before mixing the 2 ingredients, bean coats were dehulled by boiling the weighted mung beans in 10 g/L $NaHCO_3$ solution, and chestnut kernels were treated with color protection solution for several minutes (Zhang et al., 2012). Then they were mixed to continue the rest manufacturing processes including cooking, grinding, flavor blending and shoveling. 45 g refined cane sugar, 20 g syrup and 30 g vegetable oil were added into the mixture during flavor blending. DNAs extracted in triplicates from 6 simulated chestnut paste samples in weight of 50 mg were determined using duplex ddPCR and cdPCR. DNA CN ratios were calculated and substituted into the established "Mass ratio-DNA CN ratio" formulas to achieve mass ratio results. The recovery of determination is expressed as a percentage of expected mass ratio.

2.9. Detection of market chestnut paste foods

DNAs extracted in triplicates from 13 chestnut pastes and relevant market foods in Table 5 in weight of 50 mg were determined using duplex ddPCR and cdPCR. DNA CN ratios were calculated and substituted into the validated "Mass ratio-DNA CN ratio" formula to quantify mung bean contents in these samples.

3. Results and discussion

3.1. Specificity on primers and probes

The target sequences of mung bean and chestnut locate in genomes with constant copy numbers, thus are suitable for quantitative detection. The specificity of primers and probes were satisfactory in both duplex ddPCR (Fig. 1) and cdPCR (not shown in the paper). Chestnut primers and probe showed positive results to DNAs from chestnut (*Castanea mollissima*) and cone chestnut (*Castanea henryi*) thinly distributed in China, at the same time mung bean primers and probe showed positive results to mung bean DNA. No cross reaction was observed in the detection of DNAs from other relevant plant species possibly existing in paste foods.

3.2. LOQ_{copy}

Positive results were obtained for dilutions of mung bean and chestnut DNAs with a concentration as low as 1 copy/μL both in duplex ddPCR and cdPCR (Table 3 & Fig. S1~S4). Considering good precision for a quantitative detection, RSD≤25% among triplicates was set to be a key criterion (Cottenet et al., 2019). Therefore, LOQ_{copy} of mung bean and chestnut was defined as 6 copies/μL both in ddPCR and cdPCR after the numerical rounding of mean values.

3.3. Correlation between mass and DNA CN concentration

A stable and effective extraction of DNA is the first key step to ensure the accuracy of quantitative detection on biological ingredients in foods through gene contents (Dong et al., 2020). A successful DNA extraction method should be able to achieve a significant correlation between sample mass and extracted DNA amount. Therefore, correlation analysis on mass and DNA CN concentration was applied for the assessment of DNA extraction. 4 commercial DNA extraction kits were involved in the research as candidates (Table S1). Based on the performance results in Fig. 2, which indicated a satisfied linear positive correlation ($R^2 > 0.99$) between sample mass and DNA CN concentration in a sample range from 5 mg to 50 mg, Promega was confirmed to be the suitable kit.

3.4. "Mass ratio-DNA CN ratio" formula

Mung beans are always dehulled before adulteration for a better taste in industry. 2 sets of spiked samples were prepared to clarify the influence of dehulling process on quantitative detection. CN concentrations of 2 sets of spiked samples were determined using the established duplex ddPCR and cdPCR, then DNA CN ratios of mung bean were calculated from CN concentrations, and the linear Mass ratio-DNA CN ratio formulas were finally drawn using LOG_{10} (Mass ratio) as *x*-axis and DNA CN ratio as *y*-axis in Table S2~S5. Therefore, 2 sets of formulas were obtained based on whole seeds (Fig. 3) and dehulled seeds (Fig. 4) separately. To keep R^2 above 0.99, results of 1% spiked samples were not included in the formulas, thus the quantification range of the formulas was identified as 5%–80%.

Subsequently 6 simulated chestnut paste samples with a series of mung bean mass ratio contents were detected to validate the performance of 2 sets of formulas. DNA CN ratios of 6 simulated chestnut paste samples were calculated using their CN concentration determination results and substituted into the established "Mass ratio-DNA CN ratio" formulas to achieve mass ratio results. The deviation between mass ratio results calculated using 2 sets of formulas and the real contents expressed by recovery was shown in Table 4.

Considering good accuracy for a quantitative detection, recovery in the range of 80%–120% was set to be a key criterion (Mayer et al., 2019). For the formulas based on whole seeds, chestnut paste samples with mung bean mass ratios in the range of 5%–60% had unsatisfied results with the recovery from 15.40% to 71.67%. It is indicated that the

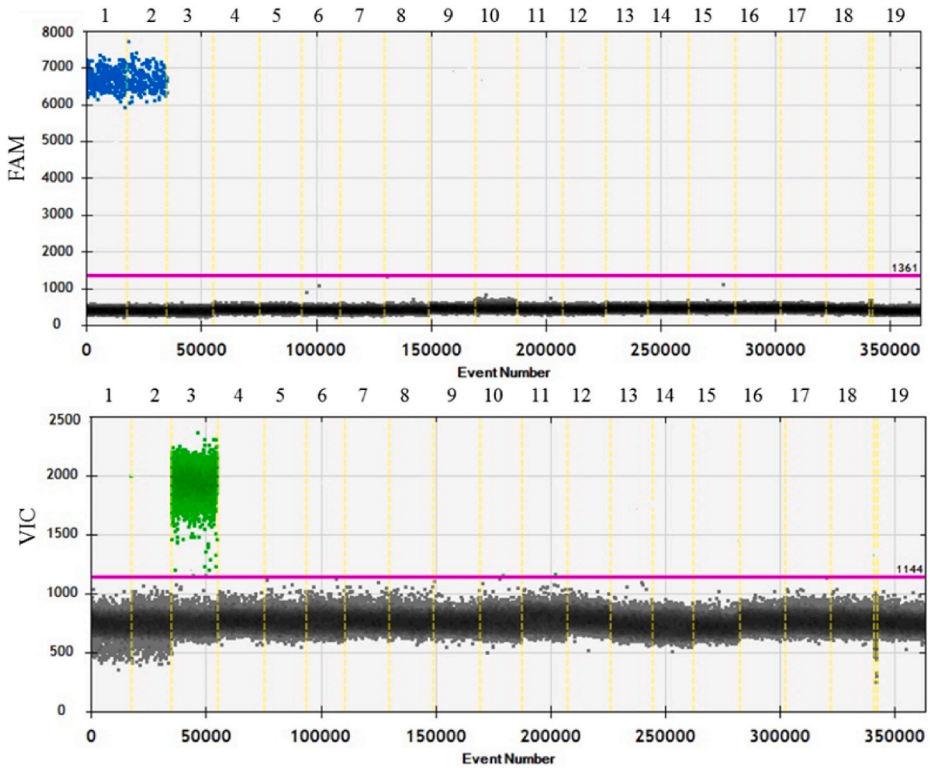

Fig. 1. Specificity on primers and probes in duplex ddPCR. 1: Chestnut; 2: Cone chestnut; 3: Mung bean; 4: Adzuki bean; 5: Rice bean; 6: Soybean; 7: Cowpea; 8: Pouch bean; 9: Kidney bean; 10: Lotus seed; 11: Hazelnut; 12: Peanut; 13: Gorgon ruit; 14: Coix seed; 15: Rice; 16: Wheat; 17: Maize; 18: Chinese yam; 19: non-template control.

Table 3
Limits of quantification for DNA CN concentration (LOQ_{copy}).

dPCR	Gradient dilution of DNA/copies/μL	Chestnut/ copies/μL Mean ± SD	RSD/ %	Mung bean/ copies/μL Mean ± SD	RSD/ %
ddPCR	100	100.87 ± 6.28	6.22	95.67 ± 3.21	3.36
	50	50.93 ± 1.35	2.65	49.37 ± 0.91	1.84
	20	21.10 ± 2.16	10.25	19.50 ± 0.87	4.47
	10	9.68 ± 0.69	7.12	9.97 ± 0.90	8.99
	5	5.27 ± 0.68	12.92	5.68 ± 0.49	8.54
	1	1.03 ± 0.55	53.52	1.30 ± 0.46	35.25
cdPCR	100	100.53 ± 3.93	3.91	104.23 ± 4.53	4.35
	50	51.10 ± 1.85	3.62	48.15 ± 1.63	3.38
	20	19.83 ± 1.14	5.73	19.43 ± 0.91	4.67
	10	10.53 ± 0.81	7.67	9.50 ± 0.79	8.36
	5	5.57 ± 0.50	9.04	5.58 ± 0.56	10.07
	1	1.06 ± 0.43	40.04	1.25 ± 0.44	35.55

formula based on dehulled beans is suitable for a quantitative detection of mung bean mass ratio content in chestnut paste.

The difficulty of calculation on mass content of biological components in deep processed foods directly from determined gene CN has been proved by many researches. The key point of mass content detection on biological components in deep processed foods through gene quantification is to build up a precise and accurate connection between gene CN and mass contents (Dong et al., 2020). The mass of DNA extracted in foods was used as a bridge to connect DNA CN with mass content in some approaches, where two standard curves were established from DNA CN to DNA mass then from DNA mass to mass of biological component in food. Unfortunately two steps of conversion established with raw material powder could lead to significant quantitative detection errors (Cai et al., 2014). Later, the research focus turned to quantification on mass ratio rather than mass itself. The establishment of mass ratio-DNA CN ratio formulas made it possible to quantitatively detect biological components in deep processed foods, based on the assumption that different components go through the same manufacturing process and experience the same loss thus maintain a stable ratio relationship (Dong et al., 2020; Köppel et al., 2020; Ren et al., 2017).

Concerning chestnut paste adulteration with mung beans, dehulling process before the mixture of chestnuts and mung beans makes it unable to obtain an accurate quantification result if the mass ratio-DNA CN ratio formula is established using spiked DNA solutions or spiked raw material powder samples according to the previous researches (Dong et al., 2020; Ren et al., 2017). Therefore, the influence of dehulling on standard curve plotting must be involved and a simulation of mung bean dehulling following industrial adulteration process should be applied.

It is proved that a practical technical route for mass content detection of biological components in deep processed foods by means of gene determination may choose the way of mass ratio as well as involve manufacturing processes which significantly influence mass ratio during the establishment of mass ratio-DNA CN ratio formula.

3.5. Detection of market chestnut paste foods

Among 13 chestnut pastes and relevant market foods in Tables 5 and 5 samples (No. 6–10) contained no chestnut ingredient and were proved to be totally fake foods. At the same time 3 samples (No. 1, 4 & 5) contained mung bean ingredients with a mass ratio higher than 40%, which meant illegal chestnut content according to National Standard GB/T 21270–2007 in China, were proved to be adulterated foods from EMA. Detection of market chestnut foods indicated that adulteration in chestnut paste foods with mung beans is very common in the market.

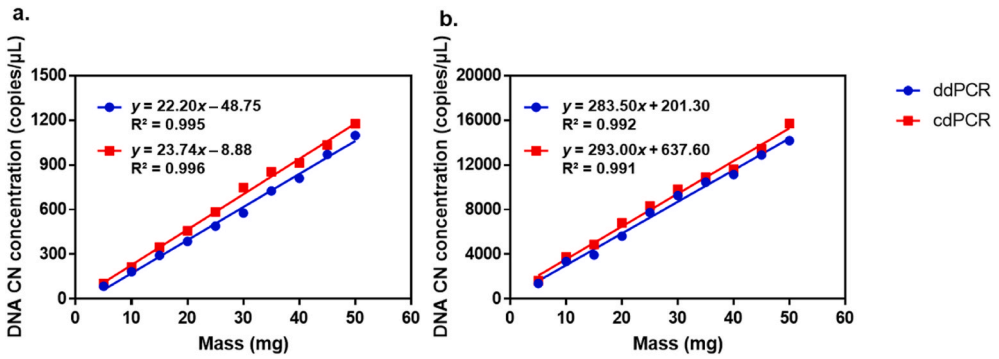

Fig. 2. Linear fitting curves of mass-DNA CN concentration. The standard curves for ddPCR and cdPCR were drawn using DNA CN concentration results from mass of 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg and 50 mg. Panel a shows the result of chestnut and panel b shows the result of mung bean.

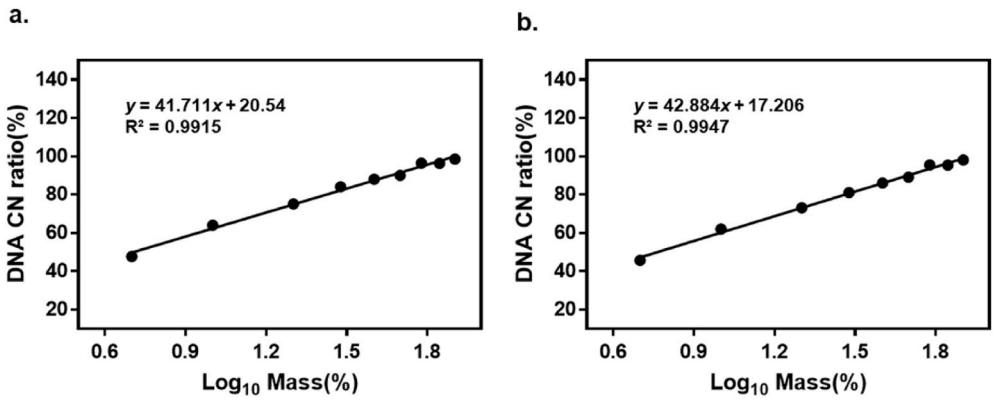

Fig. 3. Mass ratio-DNA CN ratio formula based on whole mung bean seeds. The standard curves for ddPCR and cdPCR were drawn using DNA concentration results from mung bean mass ratios of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80%. Panel a showed the result from ddPCR and panel b showed the result from cdPCR.

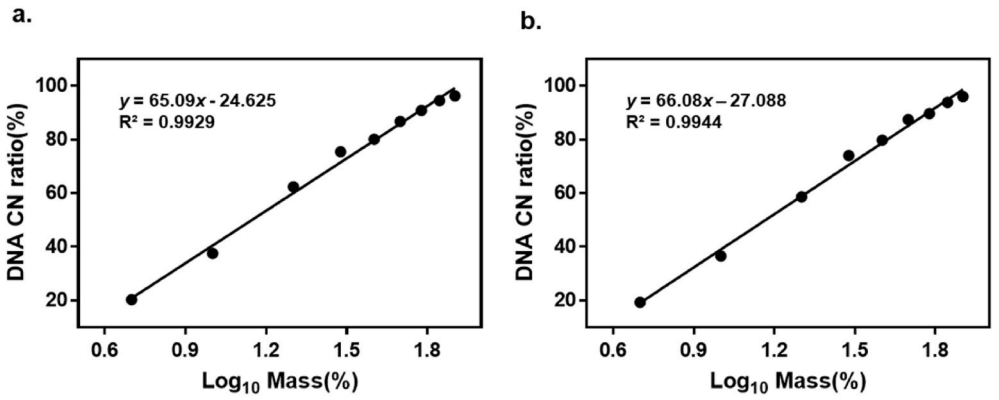

Fig. 4. Mass ratio-DNA CN ratio formula based on dehulled mung bean seeds. The standard curves for ddPCR and cdPCR were drawn using DNA concentration results from mung bean mass ratios of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80%. Panel a showed the result from ddPCR and panel b showed the result from cdPCR.

4. Conclusion

A quantitative detection method on mass ratio of mung bean in chestnut paste foods based on the determination of DNA CN ratio using duplex ddPCR and cdPCR was reported in this paper to cover the demand of identification on adulterated and fake chestnut paste foods produced by mung bean according to relevant standards in China. On the basis of previous researches of mass ratio quantification for biological components in processed foods, an improvement was achieved by involving manufacturing steps which significantly influence biological component mass in the establishment of correlation between DNA CN ratio and mass ratio. With this improvement, a better technical route of quantitative detection for deep processed foods with more complicated manufacturing processes was provided.

In fact, there are much more detection demands on authentication and quantification on biological components in processed foods besides chestnut paste. All of them have to face the same question, which is the establishment of a bridge between DNA CN concentration and mass. No matter one formula in this paper, or two formulas in some other research (Cai et al., 2014; Dong et al., 2020; Ren et al., 2017), the establishment

Table 4
Recovery of mung bean mass ratios in simulated chestnut paste samples.

Mung bean mass ratio expected	Formula based on whole mung bean seeds				Formula based on dehulled mung bean seeds			
	ddPCR		cdPCR		ddPCR		cdPCR	
	Mass ratio/%	Recovery/%	Mass ratio/%	Recovery/%	Mass ratio/%	Recovery/%	Mass ratio/%	Recovery/%
5%	0.77 ± 0.05	15.40 ± 0.92	0.88 ± 0.04	17.58 ± 0.73	4.18 ± 0.16	83.59 ± 3.20	4.30 ± 0.12	86.07 ± 2.34
10%	2.26 ± 0.13	22.63 ± 1.25	2.50 ± 0.15	25.05 ± 1.49	8.34 ± 0.30	83.38 ± 2.98	8.49 ± 0.33	84.92 ± 3.27
20%	8.61 ± 0.46	43.04 ± 2.29	8.13 ± 1.04	40.66 ± 5.21	19.63 ± 0.67	98.16 ± 3.33	18.22 ± 1.50	91.08 ± 7.51
40%	25.64 ± 1.50	64.10 ± 3.75	24.95 ± 0.84	62.38 ± 2.09	39.51 ± 1.49	98.78 ± 3.72	37.76 ± 0.82	94.39 ± 2.05
60%	40.15 ± 2.44	66.92 ± 4.07	43.00 ± 2.26	71.67 ± 3.76	52.67 ± 2.05	87.74 ± 3.41	53.74 ± 1.83	89.57 ± 3.05
80%	64.85 ± 0.24	81.06 ± 0.30	69.20 ± 0.74	86.50 ± 0.93	71.63 ± 0.17	89.54 ± 0.21	73.20 ± 0.51	91.50 ± 0.64

Table 5
Detection of market chestnut paste foods.

No.	Sample names	Ingredients on label	Mung bean mass ratio (%)		
			Expected values	ddPCR results	cdPCR results
1	Chestnut paste1	Chestnut	0	68.79 ± 0.11	67.98 ± 0.54
2	Chestnut paste2	Chestnut	0	0	0
3	Chestnut paste3	Chestnut	0	0	0
4	Chestnut pie1	Chestnut and mung bean	<40	>80	>80
5	Chestnut pie2	Chestnut and mung bean	<40	76.11 ± 1.20	77.69 ± 0.47
6	Chestnut pie3	Chestnut and mung bean	<40	100	100
7	Chestnut pie4	Chestnut	0	100	100
8	Chestnut pie5	Chestnut	0	100	100
9	Chestnut pie6	Chestnut	0	100	100
10	Chestnut pie7	Chestnut paste	0	100	100
11	Chestnut pie8	Chestnut	0	0	0
12	Chestnut flavor pie1	Dehulled mung bean	100	100	100
13	Chestnut flavor pie2	mung bean	100	100	100

of the bridge is still so far from the complete solution for mass quantification of biological component in processed foods and the real application of these techniques. Therefore, it is vitally important to have much more research and discussion on methodology.

CRediT authorship contribution statement

Yingjie Liang: Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft. **Dongwei Gao:** Validation, Writing – original draft. **Jie Dong:** Methodology, Supervision. **Lijun Guan:** Resources, Investigation. **Zhiyong Li:** Project administration. **Jin Liu:** Conceptualization, Data curation, Writing – review & editing.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this manuscript.

We would like to declare that the work described was original research and has not been published previously, and not under consideration for publication elsewhere.

Acknowledgments

We would like to thank anonymous reviewers. This work was financially supported by 2017 Guangzhou Science & Technology Project 'A Research and Application of Digital PCR Quantitative Detection Technology for Food Authentication' (Project No. 201704030125), 2017 Guangdong Provincial Science & Technology Project 'The Construction of Quantification Platform and Genomic Identification Database for Animal and Plant Derived Ingredient Adulteration in Foods' (Project No. 2017B020207008), and 2017 Guangzhou Customs Science & Technology Project 'A Research of Digital PCR Quantitative Detection Technology for Plant Derived Materials in Paste Fillings of Bakery Foods' (Project No. 2017GDK44). In addition, Weng Xiaorong, Chen Yunqi and Dong Xuwan helped a lot while preparing the simulated samples using in this work, for which the authors are grateful.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2021.12.003.

References

Baer, U., Bozolu, M., Erolu, N.A., Topuz, B.K., 2018. Forecasting chestnut production and export of Turkey using ARIMA model. Turkish J. Forecast. 2 (2), 27–33. https://doi.org/10.34110/forecasting.482789.

Baker, M., 2012. Digital PCR hits its stride. Nat. Methods 9 (6), 541–544. https://doi.org/10.1038/nmeth.2027.

Blaiotta, G., Capua, M.D., Coppola, R., Aponte, M., 2012. Production of fermented chestnut purees by lactic acid bacteria. Int. J. Food Microbiol. 158 (3), 195–202. https://doi.org/10.1016/j.ijfoodmicro.2012.07.012.

Cai, Y., Li, X., Lv, R., Yang, J., Li, J., He, Y., Pan, L., 2014. Quantitative analysis of pork and chicken products by droplet digital PCR. BioMed Res. Int. 810209 https://doi.org/10.1155/2014/810209.

Cottenet, G., Blancpain, C., Chuah, P.F., 2019. Performance assessment of digital PCR for the quantification of GM-maize and GM-soya events. Anal. Bioanal. Chem. 411 (11), 2461–2469. https://doi.org/10.1007/s00216-019-01692-7.

Dingle, T.C., Sedlak, R.H., Cook, L., Jerome, K.R., 2013. Tolerance of droplet-digital PCR vs real-time quantitative PCR to inhibitory substances. Clin. Chem. 59, 1670–1672. https://doi.org/10.1373/clinchem.2013.211045.

Dong, X., Gao, D., Dong, J., Chen, W., Li, Z., Wang, J., Liu, J., 2020. Mass ratio quantitative detection for kidney bean in lotus seed paste using duplex droplet digital PCR and chip digital PCR. Anal. Bioanal. Chem. 412 (7), 1701–1707. https://doi.org/10.1007/s00216-020-02410-4.

El Sheikha, A.F., 2019. DNAFoil: novel technology for the rapid detection of food adulteration. Trends Food Sci. Technol. 86, 544–552. https://doi.org/10.1016/j.tifs.2018.11.012.

Everstine, K., Spink, J., Kennedy, S., 2013. Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. J. Food Protect. 76 (4), 723–735. https://doi.org/10.4315/0362-028X.JFP-12-399.

Floren, C., Wiedemann, I., Brenig, B., Schütz, E., Beck, J., 2015. Species identification and quantification in meat and meat products using droplet digital PCR (ddPCR). Food Chem. 173, 1054–1058. https://doi.org/10.1016/j.foodchem.2014.10.138.

Hindson, C.M., Chevillet, J.R., Briggs, H.A., Gallichotte, E.N., Ruf, I.K., Hindson, B.J., Vessella, R.L., Tewari, M., 2013. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat. Methods 10 (10), 1003–1005. https://doi.org/10.1038/nmeth.2633.

Hsieh, Y.-H.P., Ofori, J.A., 2014. Detection of horse meat contamination in raw and heat-processed meat products. J. Agric. Food Chem. 62 (52), 12536–12544. https://doi.org/10.1021/jf504032j.

Köppel, R., Ledermann, R., Velsen, F.V., Ganeshan, A., Guertler, P., 2020. Duplex digital droplet PCR for the determination of apricot kernels in marzipan. Eur. Food Res. Technol. 246 (12), 965–970. https://doi.org/10.1007/s00217-020-03463-6.

Li, Q., Shi, X., Zhao, Q., Cui, Y., Ouyang, J., Xu, F., 2016. Effect of cooking methods on nutritional quality and volatile compounds of Chinese chestnut (Castanea mollissima Blume). Food Chem. 201, 80–86. https://doi.org/10.1016/j.foodchem.2016.01.068.

Mayer, W., Schuller, M., Viehauser, M.C., Hochegger, R., 2019. Quantification of the allergen soy (Glycine max) in food using digital droplet PCR (ddPCR). Eur. Food Res. Technol. 245 (2), 499–509. https://doi.org/10.1007/s00217-018-3182-5.

Mubarak, A.E., 2005. Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. Food Chem. 89 (4), 489–495. https://doi.org/10.1016/j.foodchem.2004.01.007.

Quan, P.-L., Sauzade, M., Brouzes, E., 2018. dPCR: a technology review. Sensors 18 (4), 1271. https://doi.org/10.3390/s18041271.

Ren, J., Deng, T., Huang, W., Chen, Y., Ge, Y., 2017. A digital PCR method for identifying and quantifying adulteration of meat species in raw and processed food. PLoS One 12 (3), e0173567. https://doi.org/10.1371/journal.pone.0173567.

Scollo, F., Egea, L.A., Gentile, A., Malfa, S.L., Dorado, G., Hernandez, P., 2016. Absolute quantification of olive oil DNA by droplet digital-PCR (ddPCR): comparison of isolation and amplification methodologies. Food Chem. 213, 388–394. https://doi.org/10.1016/j.foodchem.2016.06.086.

Sun, L., Huang, W., 2017. A real-time PCR assay for rapid detection of Kidney Bean component in lotus seed paste product. Food Sci. (N. Y.) 38 (22), 330–334. https://doi.org/10.7506/spkx1002-6630-201722049.

Wan, J., Song, L., Wu, Y., Brzoska, P., Keys, D., Chen, C., Valliyodan, B., Shannon, J.G., Nguyen, H.T., 2016. Application of digital PCR in the analysis of transgenic soybean plants. Adv. Biosci. Biotechnol. 7, 403–417. https://doi.org/10.4236/abb.2016.710039.

Whale, A.S., Cowen, S., Foy, C.A., Huggett, J.F., 2013. Huggett. Methods for applying accurate digital PCR analysis on low copy DNA samples. PLoS One 8 (3), 1–10. https://doi.org/10.1371/journal.pone.0058177.

Zhang, Y., Yang, F., Chu, L., Zhao, X., Yang, D., Lu, W., 2012. Optimize the graded color protection technology of the chestnut paste by orthogonal design. Food Sci. Technol. 37 (11), 98–102. https://doi.org/10.13684/j.cnki.spkj.2012.11.051.