Detection of Porcine DNA Residue in Various of Collagen Supplements

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Abstract

Collagen is the main protein that makes the connective tissue structure of vertebrates and can be found in interstitial tissue in almost all parenchymal organs with a function as a stabilizer and maintains the shape of these organs. Collagen can be divided into three major categories based on the source, collagen from cows, pigs and marine animals. Collagen derived from cows is extracted from skin and bones, but type of collagen derived from cows is takes of concern because of BSE or mad cow problems and is also reported to cause allergies in about 3% of the population. The alternative is using collagen from pork and marine animals. However, the use of pork collagen or contamination of pork collagen is prohibited for Muslims. The aims of this study to detection the residue DNA in collagen supplements and to identified the origin of DNA residue from non-halal materials. The results obtained from whole samples have purity in the range 1.25-4, and one sample with high purity of DNA ratio is 1.91. The results testing of collagen sample using RT-PCR (qPCR) showed that the value of Cq FAM values in positive control were 30.34 and 31.89, only one sample was amplified with Cq value 12.27 that has high difference from Cq value of positive control. Cq value of VIC from whole samples ranging of 29.41-31.91 means the sample also contained vertebrates.

Keywords: collagen, DNA, porcine, RT-PCR

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INTRODUCTION

Collagen is the main protein that makes the connective tissue structure of vertebrates with a proportion of about 30% of the total protein in body. Collagen can also generally be found in interstitial tissue in almost all parenchymal organs with a function as a stabilizer and maintains the shape of these organs (Chai et al., 2010). Silvipriya et al. (2015) divided collagen sources into three major categories, i.e. collagen from cows, pigs and marine animals. Collagen derived from cows is extracted from skin and bones and is one of the largest sources of collagen for the industry, but the use of collagen derived from cows is takes of concern because of BSE or mad cow problems and is also reported to cause allergies in about 3% of the population.

Collagen derived from pork is obtained from skin and bones, this collagen is generally

used for industrial scale. However, the use of pork collagen or contamination of pork collagen is prohibited for Muslims.

Several tests using simplex and multiplex PCR are available to explain differences in the origin of proteins such as gelatin. The target of detection is testing DNA residues on proteins such as gelatin and collagen, but there are several weakness of this method, including the high cost of the testing. Sultana (2018) has conducted tests to determine the source of protein origin such as gelatin using tetraplex PCR with tests involving eukaryotic cells, fish, cattle and pig controls for simultaneous detection of species. Halal authentication is carried out on several products containing pork and pork derivatives, especially in food and cosmetic products. Analysis using DNA can be used in all cell types of an organism, and the DNA was extracted can come from muscle, fat or even the gut.

MATERIALS AND METHODS

Research materials are various of collagen supplements products. The collagen that will be analyzed is collagen supplements that have certified and not certified of halal label. Materials used for analysis are DNeasy Mericon Food for extraction the DNA, Mericon Pig Kit for Real Time-PCR, chloroform and ethanol.

Methods

A. DNA Extraction

Prepare the 200 mg of sample and add 1 mL of food lysis buffer (can be directly 1 mL or gradually 500 µL and 500 µL, depending on the type of sample). Followed by a purification process by taking 1 mL of sample and adding 2.5 proteinase-K, vortex and incubation at 60°C for 30 minutes. Centrifuge at 2500 x g for 5 minutes. Add 500 µL of chloroform to the new 2 mL tube. Remove the clear layer from the lysis tube, without touching precipitation at the bottom of the tube. Add 500 µL to the tube which contain chloroform. Vortex for 15 seconds and centrifuge at 14000 x g for 15 minutes. Take a clear layer and calculate the volume, then add 1:1 PB buffer volume and vortex for 15 seconds. Placed all liquid into the Qiaquick spin column and centrifuge at 17900 x g for 1 minute. Dispose of the liquid stored in the collection tube. Add 500 µL of Buffer AW2, centrifuge at 17900 x g for 1 minute and remove the supernatant. Place Qiaquick spin column in 2 mL of the new collection tube and centrifuge it again at 17900 x g for 1 minute on the dry membrane. Discard the collection tube and place the Qiaquick spin column in the new 1.5 mL tube. Add 150 µL of EB and incubate for 1 minute at room temperature then centrifuge for 1 minute. The eluted DNA can be directly used for PCR or stored at -20°C.

B. RT – PCR Analysis

Add the 130 µL multiplex PCR master mix into mericon assay tube, vortex and centrifuge**.** Dissolve the positive DNA, add 200 µL quantitec nucleic acid dilution buffer, vortex and centrifuge. Set up all the sample and control of reaction following the protocol.

Table 1. Set program of RT-PCR

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStartaq Plus DNA polymerase
3-step cycling: Denaturation Annealing Extension	15 s 15 s 10 s	95°C 60°C 72°C	Data collection at 60°C
Number of cycles	45		
Detection	Reporter	Excitation/ emission	Channel
Target	FAM	495/520 nm	Green
Internal Control	VIC	524/557 nm	Yellow

Set program of RT-PCR and insert the plate containing the sample and control into the machine then run according to the RT-PCR program.

RESULTS AND DISCUSSION

A. Extraction of DNA

In this study, all the samples was isolated by DNEasy Mericon Kit, then analyzed the purity using Nanodrop Spectrophotometer. DNA purity can be identified using measurements at 260 and 280 wavelengths, and the ratio value between 1.8-

2.0 which indicates that the absorption of UV light in nucleic acids (Esa, 2018). The results obtained from whole samples have purity in the range 1.25-4, and one sample with high purity of DNA ratio is 1.91.

Table 2. Extraction result of collagen

	260	280	Ratio
C1	0.0506	0.0465	1.25
C2	0.0491	0.0466	1.71
C3	0.0523	0.0486	4
C4	0.0541	0.0506	1.37
C5	0.0726	0.0635	1.56
C6	0.0655	0.0574	1.6
C7	0.3572	0.2565	1.54
C8	0.0535	0.049	1.69
C9	0.0739	0.0644	1.53
C10	0.1006	0.0799	1.54
C11	0.0561	0.052	1.7
C12	0.06	0.0544	1.54
C13	0.0496	0.047	1.58
C14	0.0565	0.0526	2.36
C15	0.069	0.0624	1.5
C16	0.0806	0.0721	1.91
C17	0.0555	0.0523	1.33
C18	0.0533	0.0494	1.48
C19	0.0543	0.0504	1.56
C20	0.0744	0.0655	1.53

According to Pachchigar et al. (2016), ratio of DNA purity value below 1.8 indicates that there is still protein contamination, while a purity value above 2.0 indicates RNA contamination. In this study, almost whole samples extracted from collagen supplements have a ratio below 1.8, which indicates that DNA is still contaminated with protein collagen.

B. RT-PCR Analysis

The sample of DNA analyzed by RT-PCR (qPCR), the result can be seen in Table 3.

Table 3. RT-PCR of collagen suplements

	VIC	FAM	RT-PCR
C+	28.51 ; 30.71	30.34 ; 31.89	+
C-	30.12 ;30.28	–	–
C1	30.36	N/A	–
C2	30.86	N/A	–
C3	30.13	N/A	–
C4	30.28	N/A	–
C5	30.42	N/A	–
C6	30.61	N/A	–
C7	30.62	N/A	–
C8	30.62	N/A	–
C9	30.21	N/A	–
C10	30.08	N/A	–
C11	29.46	N/A	–
C12	30.16	N/A	–
C13	29.88	N/A	–
C14	30.36	N/A	–
C15	30.91	N/A	–
C16	31.91	12.27	–
C17	31.43	N/A	–
C18	30.70	N/A	–
C19	29.41	N/A	–
C20	29.75	N/A	–

The results testing of collagen sample using RT-PCR (qPCR) showed that there were amplified samples in the presence of the Quantification Cycle (Cq) value and unamplified samples (N/A). Positive control shows valid results according to the recommendations of the kit, with the value of Cq FAM is <35 or ±30 and if the Cq value between 35-45 means the sample is in the gray zone and requires re-testing. In this study, the Cq FAM values in positive control were 30.34 and 31.89. In FAM detection, the 20 samples tested, only one sample was amplified with Cq value that has high difference from Cq value of positive control.

According to Widayat et al. (2019), a positive sample containing pig DNA has Cq FAM value that is almost the same as a positive control, where the positive control has a Cq value of 33.31 and the Cq value in the positive sample is 34.60-38.23 while the negative sample does not have Cq value. In this study, the Cq value detected in one of the samples did not indicate that the sample was positive containing pig DNA, due to the low Cq value of 12.27, while referring to the kit protocol used the sample could be categorized as positive in pig if it had Cq value of ±30. The whole samples also contained vertebrates with a Cq value ranging of 29.41-31.91. According to Cahyaningsari (2019), which used the concentration of livestock pork and wild boar

in a mixture of beef in the form of raw and processed meatball up to 0.125%, produces Cq value of meatball products ranging between 29-32. RT-PCR methods can be used to detect species of animal origin because they are able to detect at concentrations less than 2%.

CONCLUSION

RT-PCR (qPCR) is the main testing method for species that have high sensitivity and specificity, as well as a short time. In this study, all samples were indicated to be negative for pig DNA, but all samples contained vertebrates. The Cq value detected in one of the samples does not indicate that the sample is positive for pork DNA, due to the low Cq value of 12.27.

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