NEW APPROACH TO GENERATE MEAT COLOR IN MEAT PROCESSING

CÁCH TIẾP CẬN MỚI ĐỂ TẠO RA MÀU THỊT TRONG CHẾ BIẾN THỊT

Chau Thanh Tuan
Soctrang Vocational College, Vietnam

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ABSTRACT

Color is one of the most important factors of sensory quality of meat products to attract consumers. In meat and meat products, myoglobin compounds in a natural form or ligand with other additives mainly contribute the pigments. Nitrosomyoglobin is the main pigment of cooked ham, while Zn-protoporphyrin (ZnPP) is that of dry-cured ham. The nitrite related myoglobin is not referred because of the cancer risks of created nitrosamines. The latter is safe but it needs a long period to develop in the dry-cured ham and is believed that related to heme and the metal core of the heme. Therefore, variant studies was carried out to find factors that can enhance the formation of ZnPP *in not only the hams but also others products.*

Keywords: *cooked ham; dry-cured ham; FECH; pigment; Zn-protoporphyrin.*

TÓM TẮT

Màu sắc là một trong những chỉ tiêu cảm quan quan trọng đối với sản phẩm thịt nhằm hấp dẫn người tiêu dùng. Trong chế biến thịt, myoglobin thường ở dạng tự nhiên hay liên kết với các phụ gia khác để tạo nên màu cho sản phẩm. Nitrosomyoglobin là thành phần chính tạo nên màu của các sản phẩm thịt chế biến (cooked ham), trong khi đó, Zn-protoporphyrin (ZnPP) lại là thành phần chính tạo nên màu của sản phẩm dry-cured ham. Sản phẩm được tạo màu bằng nitrosomyoglobin không được người tiêu dùng ưa chuộng vì nitrosamine sinh ra có nguy cơ gây ung thư. Dry-cured ham được wua chuộng hơn do tính an toàn, màu của dry-cured ham được cho rằng tạo thành bởi sự thay thế kim loại trong nhân heme. Do vậy, rất nhiều nghiên cứu đã được tiến hành để tìm các yếu tố tăng cường quá trình tạo thành ZnPP không chỉ cho sản phẩm ham nói trên mà có thể ứng dụng cho các sản phẩm khác.

Từ khóa: *cooked ham; dry-cured ham; FECH; pigment; Zn-protoporphyrin.*

1. INTRODUCTION

1.1 Dry-cured Ham

Dry-cured ham is a traditional product of meat and has a large consumption in variant countries, especially in the Mediterranean. Previously, the ham was usually made from the end of November that is the beginning of the chilling time of winter. However, nowadays, the ham was produced at any time [1], and they were salted by rubbing salt on the surface and left for a couple of weeks in the cold.

All over the world, these are several types of hams depending on the genetics, types of feed, rearing conditions of the pigs, processing conditions, and the region of origin. The European Union protects these hams by giving differences labels – Protected of Designation of Origin (PDO), Protected of

Geographical Indicator (PGI), or Traditional Speciality Guaranteed (TSG) Based on the region of origin, the hams were classified to four typical kinds, hams of the Mediterranean Area, hams of Northern Europe, hams of America, hams of China.

Hams in the Mediterranean Area have the three typical types. Spanish hams with Iberian and Serrano hams, the former was usually made from free reared Iberian heavy purred or crossed with Duroc pigs. The processing period can take 2 years or longer [1]. The Serrano ham was made from standard light pigs mostly intensively reared. These hams are produced from 6 months to 2 years with reserve, gran reserve and bodega levels. The other is Corsican ham produced in Corsica (France) from autochthonous heavy pigs. The ham can make up to 18 months. The last one is Parma ham made in the northeast of Italy. Pigs used for ham processing have live weight about 160 kg, and the raw materials are carefully controlled. The hams are processed with at least 12 months or longer, and its quality is protected by PDO.

Hams in Northern Europe are commonly processed for shorter period than those of Mediterranean, and they are usually smoked or cooked before consumption. Some kinds of them in Norway were made from not only pigs but also lambs and are ripped for 12 months or more [2]. Other types of traditional hams of the region are German Westphalian ham, Katenschinken (cold smoked ham), and Finnish “sauna” hams.

Hams in America as country style hams or country hams are salted and post salted. Then, they were incubated for around a month. Commonly, they are consumed after smoking or cooking by frying, baking [3] or roasting. Hams are mostly produced in Kentucky and Virginia, and they are also made in some others, Tennessee, North Carolina, Pennsylvania and Missouri [3].

Ham products were also produced in other places especially in China. A kind of typical hams in China is the traditional product of Jinhua district, Jinhua ham, located in the highland region of China [4]. Other kinds of hams were Xuanwei hams and Rugao hams, products of Yunnan and Jiangsu provinces, respectively.

1.2 Colors of meats and meat products

Colors of meat consist of myoglobin, hemoglobin, cytochrome c and other heme proteins in bone marrow. Among them, myoglobin is the main component of meat pigments [5]. Variant studies were carried out to maintain the natural red pigment of meat after post-slaughter, reservation, processing and shelf life period. To do that, some additives was added to meat to keep a natural form of myoglobin, or the colors of meat are contributed due to the form and ligand of iron in myoglobin with other compounds [5]. Deoxymyoglobin with iron in ferrous form in a non-oxygen condition contribute the purplish red for meat, while that of iron in the appearance of oxygen cause the bright red color of oxymyoglobin in meat. Besides, in oxidation process, deoxymyoglobin and oxymyoglobin can be converted to metmyoglobin and turn to the dark red myoglobin. In addition, myoglobin can contribute other colors for meat by the bind with other compound CO, CO_2 or NO_2 to generate corresponding carboxymyoglobin or nitrosomyoglobin, stable bright to dark red. Carboxymyoglobin is usually found in smoked meat products, while nitrosomyoglobin is found in variant cooked ham products.

Nitrosomyoglobin is very stable and commonly created by the ligand of iron with NO under heat treatment [6-7]. Therefore, to

generate to bright red colors for the cooked ham, sodium nitrate was added to the meat with the legal limited level of 150 mg/kg (sodium nitrate/meat), and then the products was boiled, steamed or smoked to increase the bright red color of the hams. Moreover, the addition of sodium nitrate into meat products is not only the color generated additive but also the preserved one by the ability of inhibit the development of spoil bacteria [1]. However, several finding found that nitrosamines, a cancer risk compound, could be generated in the cooked ham. Therefore, the ham is not be referred by consumers.

Recently, several authors paid attention to another kind of myoglobin relating colors in the Parma ham, which is made by only the incubation of meat and NaCl. The red pigment of the ham is safe for consumer and very stable in high temperature and shows fluorescent profiles (**Figure 1**). Some author tried to enhance the red pigment of dry-cured ham and the others found some methods to monitoring the quality of the ham color during long process of incubation [8]. However, the main component of the pigment had been being unknown until the finding of a Japanese [9-10], showing that ZnPP contributes of the bright red pigment, and the other reported that protoporphyrin is also contribute the red pigment of Parma ham [11]. Then, the cause of ZnPP in dry-cured ham has been the main question.

Very early researches [12] reported that bacteria could be the main cause of the formation of bright red and unknown stable pigment of dry-cured ham. Recently, some others authors believed that endogenous enzyme or bacteria can be the main cause of ZnPP formation [9,11,13-16] via three possible pathways (**Figure 2**); first, the insertion of zinc into protoporphyrin in both enzymatic and non-enzymatic reactions (**Figure 2**, pathway-2); second, the substitution of ferrous by zinc ions to heme to form ZnPP (**Figure 2**, pathway-4). Third, protoporphyrin, formed by the oxidation of protoporphyrinogen under the effect of proto-gen oxidase, is inserted zinc into porphyrin rings to form ZnPP (**Figure 2**, pathway-5). In the previous study, Our group [11] clearly showed that ferrochelatase is the main cause of the formation of ZnPP and protoporphyrin in dry-cured ham via the replacement of ferrous by zinc in anaerobic conditions in pathways named iron removal and conversion reactions, respectively (**Figure 2**, pathway-3 and 4). Since then, the ability of FECH in enhancing the pigment of the hams has been noticed by several authors around the world.

1.3 Ferrochelatase

Figure 2 showed the Pathways of ZnPP and protoporphyrin formation including the last steps of heme biosynthesis, the formation of ZnPP in case of lead poisoning (in vivo), and the enzymatic and non-enzymatic reactions of zinc insertion to determine FECH activity (in vitro), the formation of protoporphyrin via iron-removal reaction (in vivo and in vitro), the conversion reaction of heme to ZnPP via the reverse reaction, the formation of ZnPP via the oxidation of protoporphynogen. At the terminal step of heme biosynthesis, FECH (EC 4.99.1.1) catalyzes the insertion of Fe^{2+} into protoporphyrin to form protoheme (Figure 2, pathway 1) [17]. The enzyme can be found in several species microorganisms, plant and animals with a molecular weight about 33 – 42 kDa. It is located on the inner and outer the membrane of the mitochondria of animals [17,18]. The enzyme is usually in an active homodimer form [19-20]. Because of sable properties of ZnPP, zinc ions are commonly used to insert into protoporphyrin, and the

formed ZnPP is measured to calculate the FECH activity. FECH only catalyzes the insertion of zinc ions into protoporphyrin to form Zn-PP in case of lead poisoning in vivo (Figure 1, pathway-2).

The mammalian enzyme composes of iron-sulfur cluster as a functional group [19,21]. The role of this cluster in FECH activity is now unclear. This cluster is destroyed easily by the effect of several factors including temperature, reductants and reactive gas. Therefore, it causes the lose activity of FECH rapidly. DNA of FECH from many species was sequenced, and the recombinant FECH from human, bovine, bacteria and plants were successfully made and characterized [17,20]. They showed the similar chelating activity, expect for the variances of reaction rate and some optimum conditions for reaction.

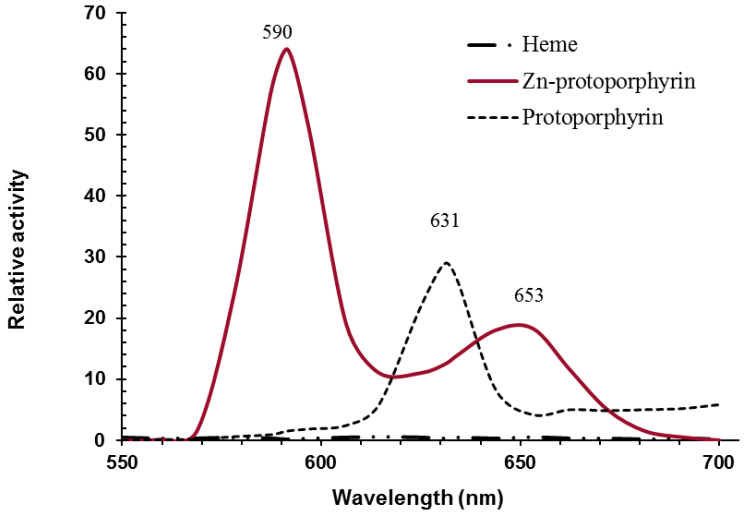

Figure 1. Fluorescent profiles of protoporphyrin and ZnPP: *ZnPP, protoporphyrin, and hemin were dissolved in acetone/ethanol (1:1, v/v). The fluorescent spectrum was observed by scanning emission from 550 nm to 670 nm with excitation at 400 nm. ZnPP has α and β peaks at 590 and 653 nm, respectively. Protoporphyrin showed a peak at 631 nm, and hemin does not show any fluorescence.*

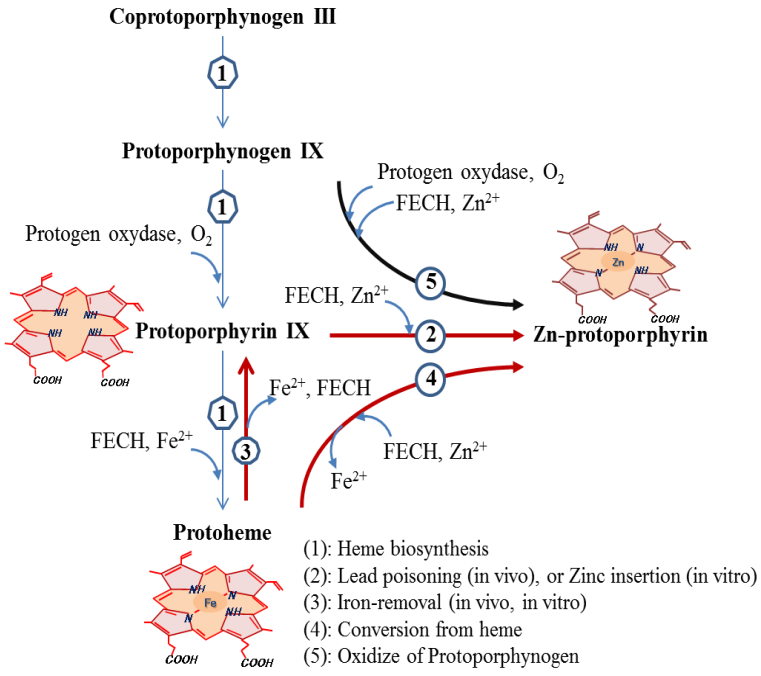

Figure 2. *Pathways of ZnPP and protoporphyrin formation*: (1) Last steps of heme biosynthesis. (2) The formation of ZnPP in case of lead poisoning (in vivo), and the enzymatic and non-enzymatic reactions of zinc insertion to determine FECH activity (in vitro). (3) The formation of protoporphyrin via iron-removal reaction (in vivo and in vitro). (4) The conversion reaction of heme to ZnPP via the reverse reaction. (5] The formation of ZnPP via the oxidation of protoporphynogen.

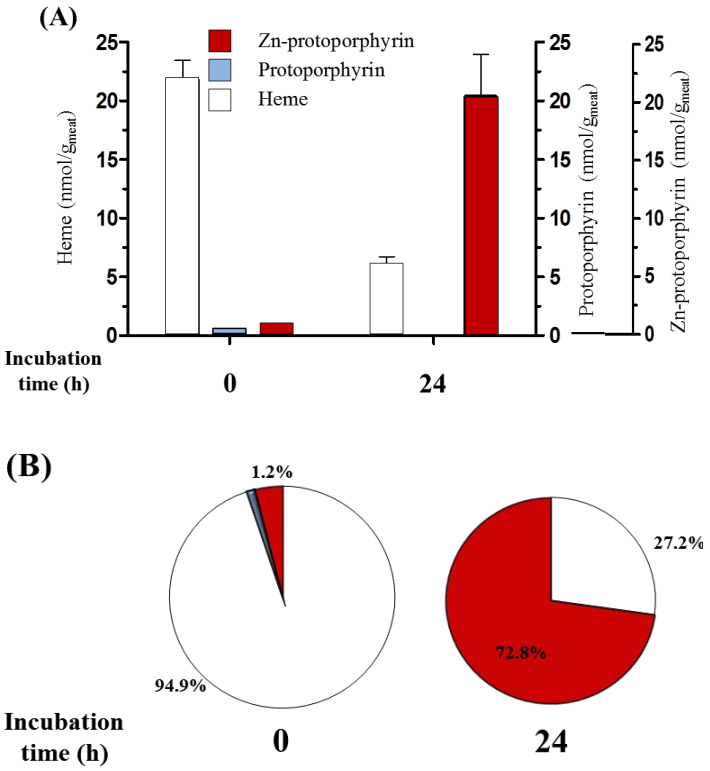

Figure 3. Proportion of metalloporphyrins and protoporphyrin in porcine muscle by the incubation with yeast FECH: *(A) The formation of Zn-protoporphyrin from heme in meat with yeast FECH. The reaction mixture containing 1 g of meat (porcine muscle), 6 mM ascorbic acid, 0.3 µg of yeast FECH, and 10mMpotassium phosphate buffer (pH 6.5) was incubated at 30 C for 24 h. The contents of ZnPP, protoporphyrin, and heme were examined. Data are expressed as means ±SDs of 24 independent experiments. (B) Change in the composition of heme, protoporphyrin and Zn-protoporphyrin after the incubation.*

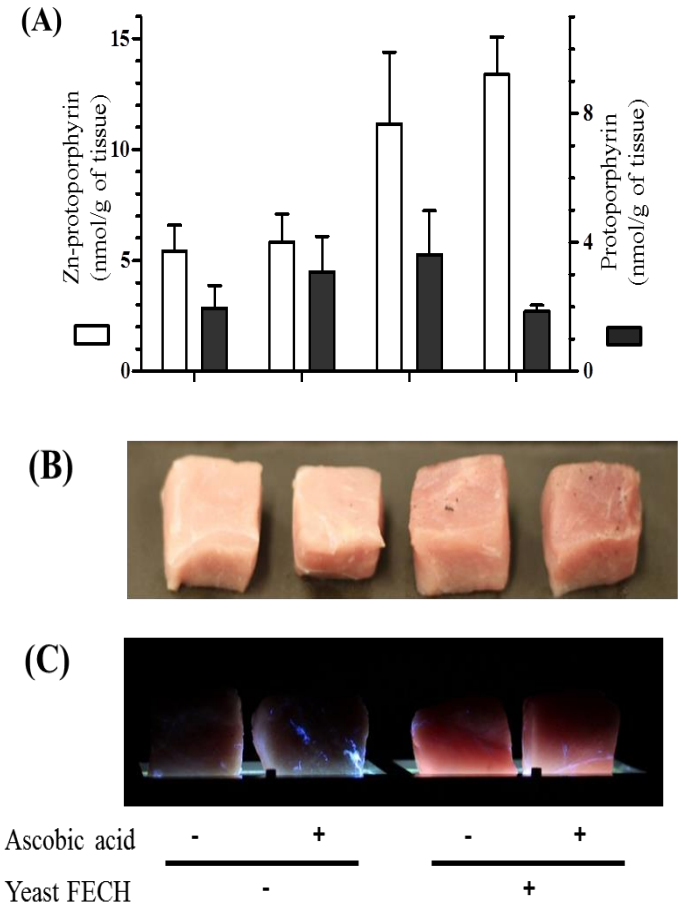

Figure 4. *Conversion of heme in meat to ZnPP using the intact piece of porcine muscle*: (A) The formation of ZnPP from heme in tissues of porcine muscle. The solution (1.0 mL) containing 1 µg of yeast FECH, 6 mM ascorbic acid, and 10 mM potassium phosphate buffer, pH 6.5, was injected into porcine muscle (15 g). The meat was incubated anaerobically at 30 ^{o}C for 24 h. After incubation, ZnPP and protoporphyrin were extracted from meat, using acetone/ethanol (1:1 v/v), and determined fluorophotometrically. Data are expressed as means ±SDs of triplicate experiments. Intact pieces of porcine muscle after incubation were exposed to white light (B) or UV light in a dark room (C), and then, the images were observed. The pink color indicates the production of ZnPP.

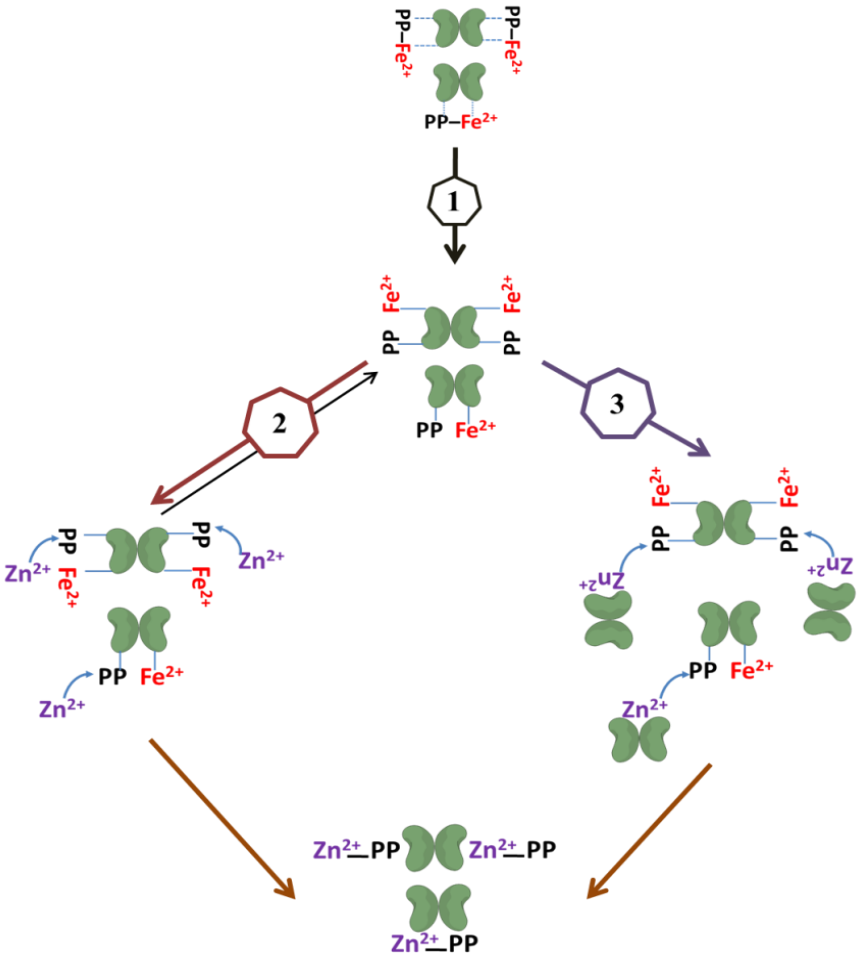

Figure 5. *Possible pathways of conversion reaction of heme to ZnPP*

1.4 Application of Enzymes in Meat Processing

Enzyme technology has been applied onto meat processing by the demand of high quality of meat products and upgrade lower quality meats. Commonly, enzymes can boost the manufacturing processing and upgrade poor quality meats by affect to the tender, flavors, and structure of meats [22]. Protease, lipase, transglutaminase, oxidative enzymes and glutaminase are the most common enzymes applied in meat manufacturing.

Protease and peptidase, proteolytic enzymes, classified as serine, cysteine aspartic, and metallo protease, are used for hydrolysis muscle in meat to tenderize [22]. Lipase can enhance the flavor formation in sausage production by conversion of triacylglycerols to soluble fatty acids and di- or monoacylglycerols. It can be from various organisms including animals, plants, fungi and bacteria. Transglutaminase and oxidative enzymes were used as the structure engineering factors in meat producing. Glutaminase enzyme can act as the flavor-enhancing factor. The enzyme can catalyze the deamindation of *L*-glutamine to *L*-glutamic acid.

In meat production, nearly no enzyme technology had been used to enhance the color formation of meat products. In this study, the roles of FECH in the removal of ferrous from heme and the alternative of ferrous in

heme by zinc to form ZnPP was clearly identified.

Recently, some investigators [23,24] used meat extract as a source for the formation of ZnPP. They found that the formation via a parallel of non-enzymatic and enzymatic reaction of FECH. In their study, protoporphyrin and zinc were added to the reaction mixture carried out in aerobic condition at pH 7.4. Therefore, in such case, iron removal and conversion of hem to ZnPP hardly occur, and only the insertion of metals into porphyrin rings can be occurred. However, the non-enzymatic reaction of the insertion of ferrous in to protoporphyrin only occurs in alkaline pH [25]. At pH lower than 6.5 with a present of saturated lipids such as palmitic acid or stearic acid the non-enzymatic reaction was completely terminated. Therefore, in meat (pH 5.5-6.0) containing some amount of saturated lipid, the non-enzymatic of the zinc-insertion reaction to form ZnPP could not occur, and FECH can play an important role in the formation of ZnPP.

The enzyme in either wide type or recombinant forms can show the similar characteristics. Besides, FECH and reductants under the anaerobic condition can significantly enhance the conversion of heme in meat to ZnPP that is the safe and stable pigment found in dry-cured ham. Taketani [11] and Chau et al., [16] extracted, purified and made the recombinant FECH. Then they successfully catalysed conversion reaction from heme to ZnPP. (**Figure 3, Figure 4, Figure 5**).

2. SUMMARY AND PROSPECTS

On these studies [16,26] the DNA of porcine FECH was successfully cloned (**Figure 6**).

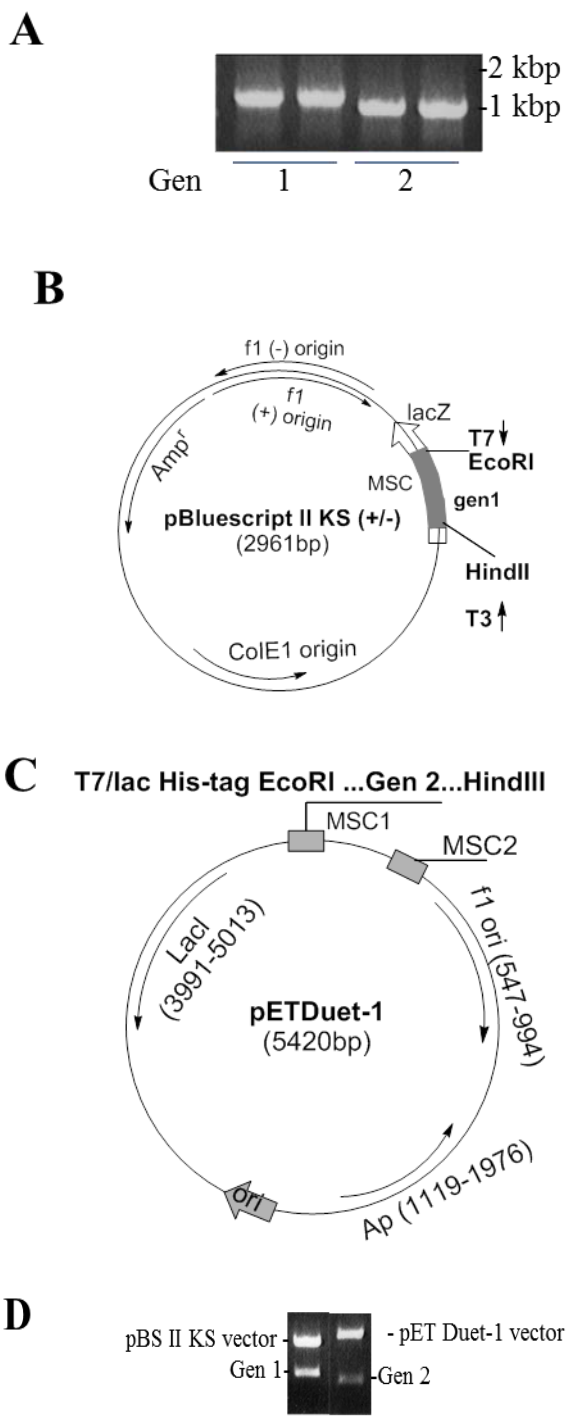

Figure 6 showed the Transformation of FECH DNAs to plasmids [16]. In here, **Figure 6*A*** showed the FECH DNA after amplified by PCR with the primers, full length (Gen 1) and expression length (Gen 2) of DNA ware loaded in 1.1% agarose gel and stained by methyl bromyl. **Figure 6B** showed the PBS II KS plasmid carrying Gen 1 of porcine FECH DNA was used for DNA

cloning. **Figure 6C** showed the PETDuet-1 plasmid carrying Gen 2 was used for expression of procine FECH. And **Figure 6D** showed the PBS II KS+ vector and Gen 1 (left), and PETDuet-1 vector and Gen 2 (right) were digested by EcoRI and HindIII in 1.1% agarose gel dyed by methyl bromyl. The relation of reverse and conversion reactions from hemin, hemoglobin, myoglobin and heme in meat, and the role of porcine FECH in the formation of ZnPP were identified. Conversion reaction requires anaerobic condition to proceed. Yeast FECH showed the highest activity and the most stable as compared to those of mammalian and bacteria. Ascorbic acid, cysteine, NADH-b_5 reductase and some lipids can promote conversion activity of the enzyme, and among them, ascorbic is the best one. However, the activities is inhibited by heavy metals. High amount of heme in meat (70%), catalyzed by FECH, can be converted to ZnPP, the bright red pigment of Parma ham, in natural pH of meat (**Figure 4, Figure 5).** These findings can open up possible methods to generate the bright red pigment of dry-cured ham as well as other meat products.

However, it has some unclear masters and some possible prospects that should be carried out further experiments to apply FECH onto pigment generation of meat products.

2.1 Identify the mechanism of the conversion reaction

The conversion reaction includes forward and reverse reactions in which the reverse reaction plays an important role. FECH removed ferrous from heme and form protoporphyrin (**Figure ,** pathway-1), after that zinc was inserted into protoporphyrin to form ZnPP via the non-enzymatic reaction by the completion of zinc ions (**Figure ,** pathway-2), or by the enzymatic reaction catalyzed by FECH (**Figure ,** pathway-3). However, up to now, it is not clear that whether the enzyme reaction or the non-enzymatic reaction is more important. Besides, how FECH interacts to heme and protoporphyrin to catalyze conversion reaction should be surveyed.

2.2 Effects of NO to the formation of ZnPP

NO decreases the formation of ZnPP in dry-cured ham and inhibits FECH activity. It is possible that NO can attack to the Fe-S cluster of FECH [21]. On the other hand, it can link to Fe^{2+} in heme of hemoproteins and block the release of the heme from hemoproteins [24]. Therefore, the substrate for the formation of ZnPP is limited.

2.3 Effects of halophilic bacteria onto conversion reaction in dry-cured ham

In dry-cured ham processing, some amount of NaCl was added to meat at some first steps [1]. In such case, halophilic bacteria could growth and produce some reductants or enzymes which may help to enhance the formation of ZnPP.

2.4 Properties of ZnPP and some effective methods to apply the conversion reaction to meat processing

ZnPP stables with heat, therefore, it can be applied onto cooked ham and can effectively generate stable colors for meat products. Suitable methods, optimize the temperature, water activity, reductants… in processing, to enhance the formation of ZnPP by FECH should be carried out.

2.5 Effects of anaerobic condition

The formation of the pigment catalyzed by FECH anaerobically, in that case, meat or reaction mixture can be spoiled easily. Therefore, the suitable conditions of

anaerobic condition should be found to reduce the spoilage of reaction systems.

2.6 Changes of sensory quality of meat products

FECH could not cause any effect to the generation of flavors (taste and aroma). However, whether the condition for the enzyme reaction of ZnPP formation could held some other factors such endogenous enzymes enhancing the formation of some volatile and aroma compounds. As a result, cause the change of ham sensory quality.

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Corresponding author:
PhD. Chau Thanh Tuan
Soctrang Vocational College, Vietnam
Email: cttuan@svc.edu.vn