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Enriching onion powder with natural nitrite for meat curing using atmospheric pressure plasma and egg whites

대기압 플라즈마와 난백을 활용한 양파 분말 내 천연 아질산 증진방안 및 육제품에의 적용

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Abstract

Enriching onion powder with natural nitrite for meat curing using atmospheric pressure plasma and egg whites

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The efficacy of egg white addition during atmospheric pressure plasma (APP) treatment on onions to replace synthetic sodium nitrite in the processed sausage was evaluated. Onions were treated with APP alone (PO) or in the presence of 30% egg whites (w/w, POE). While PO also showed an increase in nitrite content, the addition of egg white resulted in an approximately four-fold increase in nitrite content in POE compared to PO. After freeze-drying and processing into the powder form, the nitrite content of both PO and POE was concentrated well without loss. To test the practical application of this technique, four different materials of no nitrite (None), sodium nitrite (SN), PO powder, and POE powder were added to sausages then evaluated on a consumer scale. POE sausages retained a similar nitrite content, emulsion stability, and visual redness to the sausages added with sodium nitrite. Also, POE sausages achieved improved textural properties and onion like-odor, and significantly reduced warmed-over flavor. From these results, we concluded that onion powder enriched with nitrite by APP can effectively replace synthetic sodium nitrite in processed sausages without compromising their flavor.

Keywords: Atmospheric pressure plasma, Sodium nitrite, Onion, Egg white, Flavor, Sausages

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List of Abbreviations

2-AA	:	2-aminoanthracene
4-NQO	:	4-nitroquinoline-1-oxide
a^*	:	Redness
$ABTS^+$	:	2, 2'-azinobis (3-ethylbenzothiazol ine-6-sulfonic acid) radical cation
APP	:	Atmospheric pressure plasma
b^*	:	Yellowness
BC	:	Before Christ
DBD	:	Dielectric barrier discharge
DPPH	:	2, 2-diphenyl-1-picryl-hydrazyl-hydrate
L^*	:	Lightness
N_2O_3	:	Dinitrogen trioxide
N_2O_4	:	Dinitrogen tetroxide
NO	:	Nitrogen oxide
NO_2	:	Nitrogen dioxide
O	:	Fresh onion
PO	:	APP-treated onion

POE	:	APP-treated onion in the presence of egg whites
RNS	:	Reactive nitrogen species
ROS	:	Reactive oxygen species
SA	:	Sodium azide
UV	:	Ultraviolet ray

Chapter I.

Literature review

1.1. Sodium nitrite

1.1.1. History

Sodium nitrite is an inorganic compound with the chemical formula $NaNO_2$ which has a multifunctional role in processed meats (Alahakoon et al., 2015). The use of nitrite was derived from the salting for food preservation that existed before the recorded history (Keeton, 2011). In 200 BC, ancient Greeks and Romans used saltpeter (KNO_3) obtained from rocks with citrus juice to preserve meat. During this procedure, hemoglobin, myoglobin, and bacteria present in meat and ascorbic acid in citrus juice could reduce nitrate to nitrite, resulting in a curing effect on meat (Shiva et al., 2010). The mechanism of the curing effect by nitrite was not clear until the early 1900s, but nitrite has played an important role in meat curing for thousands of years (Ray, 2007). In the early 20th century, many studies were conducted on the production of sodium nitrite in a purified form for solving the problem of irregular curing and standardizing. Due to the identification of the multifunctional role of sodium nitrite and the standardization of the residual amounts in food, nitrite has become an important food additive in the food industry, especially in the meat industry (Sindelar et al., 2012).

1.1.2. Functions

1.1.2.1. Colorant

Meat color is considered to be the most important quality attribute, as it affects consumers' first impressions and purchase decisions (Yong et al., 2018a). Especially for processed meat products such as ham, sausage, and salami, consumers tend to prefer the reddish-pink color (Cornforth, 1994). Sodium nitrite can play an important role in the development of a desirable red color in meat products (Lee et al., 2019a). During the meat curing process, ionized nitrite from sodium nitrite forms nitric oxide, and this reaction can be accelerated using a reducing agent such as ascorbic acid. Subsequently, nitric oxide reacts with central iron (Fe^{+2}) in heme ring of myoglobin to form the nitrosyl-myoglobin complex (Jung et al., 2015a). Nitrosyl-myoglobin complex is a relatively unstable compound with a bright red color that can be discolored when exposed to light or oxygen. When the globin protein part is denatured during the thermal processing, nitrosyl-myoglobin is converted into nitroso-hemochrome with stable reddish-pink color, resulting in the cured meat color (Parthasarathy & Bryan, 2012).

1.1.2.2 Flavor enhancer

Nitrite is known to form characteristic flavors through a variety of chemical reactions that have not been clearly demonstrated (Alahakoon et al., 2015, Yong et al., 2018b). Since the early 1990s, many researchers have conducted a series of studies on cured meat flavor. In previous studies, uncured and cured meat showed the differences in the content of carbonyl compound (Ramarathnam et al., 1991). Cured meat was found to contain a higher level of carbonyl compounds such as hydrocarbons, ketones, alcohols, phenols, esters, furans, pyrazines, aldehydes, and carboxylic acids compared to uncured meat. In addition, higher amounts of other nitrogen containing heterocycles, sulfur, and nitrite/nitrate containing compounds were detected in cured meat than in uncured meat (Ramarathnam et al., 1993). Related sensory research suggested that characteristic cured meat flavor is a result of a cooperation between complex aroma components and flavors with an inhibited rancid flavor (Ramarathnam et al., 1998). Therefore, the results of nitrite combined with various components and aroma, through yet unknown reactions, can affect the formation of characteristic desirable flavor (Sindelar & Milkowski, 2011).

1.1.2.3. Antimicrobial effect

One of the main functions of nitrite is to inhibit the growth of microorganisms to improve food safety. Nitrite can have antimicrobial effects on food through a variety of inhibitory mechanisms. Nitrite reacts with Fe-S clusters of *Clostridium botulinum* spores to form the iron-nitric oxide complexes, thereby destroying the clusters essential for energy metabolism (Reddy & Cornforth, 1983). Through this mechanism, nitrite can inhibit the germination of *Clostridium botulinum* spores in processed meat and allow complete control over botulism, a fatal neuroparalytic food poisoning (Sindelar & Milkowski, 2011). Nitrite is also known to contribute to the growth inhibition of other anaerobic and aerobic spore-forming bacteria such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens* (Parthasarathy & Bryan, 2012, Pradhan et al., 2009). Nitrite is generally not considered effective in controlling Gram-negative bacteria such as *Salmonella* spp., and *Escherichia coli* (Davidson et al., 2005).

1.1.2.4. Antioxidant effect

In meat products, lipid oxidation is one of the significant causes of quality deterioration (Jo, 1999). Rancid odor and the warmed-over flavor are caused by lipid oxidation of meat products, and these negative sensory properties may reduce consumer acceptance. Nitrite can retard the development of oxidative rancid odor and warmed-over flavor via its antioxidant effect (Sindelar & Milkowski, 2011). This antioxidant effect is attributed to the stabilization of free radicals promoting lipid oxidation such as heme iron, metal ions, and oxygen via the reaction with nitric oxide converted from nitrite (Bergamaschi & Pizza, 2011). Therefore, the use of nitrite can improve the quality of meat products through anti-oxidant and flavor-enhancing effects.

1.1.3. Application for meat products

1.1.3.1. Meat curing

Nitrite can serve as the most important ingredient, especially in meat curing, as it plays a multi-functional role. During the meat curing, nitrite functions as a colorant, flavor enhancer, antimicrobial, and antioxidant agent, thereby resulting in giving the meat product stable red color, unique cured meat flavor, suppressing warmed-over flavor and outgrowth of *Clostridium botulinum* spores. For these reasons, nitrite has widely used in the meat industry as a useful food additive under the approval of the European Union (EU), the United States Department of Agriculture (USDA), and The Food and Drug Administration (FDA) (Merino et al., 2016).

1.1.3.2. Consumer concerns and demands

Food additives such as nitrite benefit both consumers and food processors in terms of ensuring food safety, improving sensory attributes, and reducing costs (Pașca et al., 2018). Although the safety of the use of food additives has been sufficiently discussed and proven, the addition of chemicals to food is still an ongoing issue due to the high consumer concern about the health problem and high preference for “natural” and “chemical-free” food labels. Even in the case of sodium nitrite, the use of it is controversial because nitrite can react with secondary amines in certain environments such as acidic pH conditions and high temperatures above 130°C to form carcinogenic nitrosamines (Yong et al., 2018b). However, the formation of nitrosamines via this reaction rarely occurs under normal conditions, and vitamin C is known to be able to effectively prevent nitrosamine formation. Therefore, many countries currently regulate the residual nitrite content in meat products and recommend adding vitamin C as an ingredient. Despite these regulations and risk-prevention measures, consumers demand less chemical use in meat products. To meet these consumer demands, the current meat industry is focusing on developing natural materials or natural nitrite sources that can replace sodium nitrite (Alahakoon et al., 2015; Jung et al., 2015b).

1.1.4. Natural nitrite sources

In response to consumer preference for foods without synthetic additives, many studies have been conducted on the replacement of sodium nitrite (Alahakoon et al., 2015). One of the newly developed meat curing processing is to use certain vegetables instead of sodium nitrite. Celery, beetroots, and spinach juice or powder are mainly used because they are suitable for this method due to their high nitrate content. This method also requires the process of converting nitrates into nitrites by culturing nitrate-reducing bacteria to provide a curing effect to meat products (Mowat et al., 2001). However, the use of vegetables concentrates on meat products may increase bacterial strain management and labor costs and create an off-flavor for meat products (Jung et al., 2015a). Therefore, to solve these problems, further research on the development of new substitutes of sodium nitrite or novel processes is needed.

1.2. APP and foods

1.2.1. Definition of APP

Plasma, literally defined as the fourth material state, is a state in which the gas receives high energy and contains free electrons, ions, and neutral particles, such as excited atoms and free radicals with high reactivity and UV emission ability (Kim et al., 2013). Plasma with high energy can change the properties of the material surface by contact and has a high conductivity similar to that of metal, although it is not solid or liquid. Due to these characteristics, plasma has been widely used in chemical synthesis industries such as semiconductor process, etching, and high purity silica manufacturing, and these processes have been mainly performed through a vacuum plasma method to easily generate plasma (Ryu et al., 2013).

Recently, as plasma technology has developed, APP technology that generates plasma in the air has appeared (Misra et al., 2014). This APP has the advantage that the material to be treated is relatively free from the effects of vacuum or heat, and thus can be applied to various fields such as food, biology, and medicine (Song et al., 2016). Also, since APP does not require a vacuum process, it can perform continuous processing and reduce the cost of installing vacuum equipment. For these reasons, the APP is considered to be more efficient and economical than conventional vacuum plasma (Jayasena et al., 2015).

1.2.2. Application of APP in the food industry

1.2.2.1. Microbial inactivation

In the food industry, APP has been mainly applied to improve food safety through decomposition of residual pesticides and chemicals, enzyme inactivation, and microbial control (Pankaj et al., 2017). In particular, APP is emerging as a promising non-thermal technology for sterilization of fresh foods such as fruits, vegetables, and meat, because it can effectively inactivate microorganisms without compromising the characteristics of foods, unlike traditional thermal treatment. (Yong et al., 2014; Zhang et al., 2013) Today, the antibacterial mechanism using APP is mainly based on RNS and ROS such as singlet oxygen, hydroxyl radical, peroxide anion, hydrogen peroxide, and ozone. These radicals are known to induce inactivation of microbial spores, cell apoptosis via intracellular DNA damage, and chemical transformation, and degradation of membrane proteins and lipids (Lao et al., 2017; Sensenig et al., 2011).

It has been reported that APP can induce changes in cell morphology, including cell breakage and cell leakage in *Escherichia coli* (Han et al., 2016). *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* spot-inoculated on apples, melons, and lettuce, respectively, showed a population reduction of more than 3-log for 5 min after APP treatment. (Critzer et al., 2007).

However, there is a limitation that APP is not suitable for sterilization of pre-packaged food because it has less permeability than radiation or high-temperature treatment, thus its availability is confined to surface sterilization. In recent years, many studies on in-packaging plasma systems are carried out to overcome these limitations. For example, a flexible thin-layer DBD plasma systems in the form of commercial food packages have been reported to have an antimicrobial effect on pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated with cheese and beef jerky (Yong et al., 2015a; Yong et al., 2017).

1.2.2.2. Enhancement of the activity of bioactive substances

APP induces a change in the chemical properties of the treated material, and in this process, the activity of various bioactive substances can be increased. Jeong and Kim (2019) confirmed that when APP was applied to red wine for 60 min, a large amount of trans-Resveratrol was decomposed to generate low molecular phenolic compounds such as 3,5-dihydroxybenzaldehyde and *p*-hydroxybenzaldehyde, and $ABTS^{+}$ and hydroxyl radical scavenging activity was enhanced. Yodpitak et al. (2019) reported that DBD plasma-treated brown rice had higher levels of γ-oryzanol with the ability to stabilize autonomic nerves than untreated brown rice during two days of germination. Naringin dissolved in methanol was converted to flavonoids with higher bioactivity than the original naringin after DBD plasma treatment (Kim et al., 2015). This converted new flavonoids showed improved scavenging activity against peroxynitrite and DPPH radicals. The above results indicate that APP treatment can improve the activity of bioactive substances in food and create new substances to improve the nutritional value of food.

1.2.3. Nitrite generation using APP

As consumer demand for nitrite replacement increases, many studies have been conducted to generate nitrite using the properties of APP (Ercan et al., 2016; Jung et al., 2015a). Interaction between APP and liquid may result in the production of nitrite from nitrogen oxides, such as NO, NO_2, N_2O_3, and N_2O_4 (Oehmigen et al., 2010). The generation of nitrite by APP treatment proceeds as follows. When air is discharged via APP treatment, RNS is generated from nitrogen, which can be diffused and dissolved in peripheral liquid. The dissolved nitrogen oxides have a series of reaction with water molecules, and nitric acid and nitrous acid formed through these steps are decomposed into nitrate and nitrite, respectively (Lee et al., 2017). Several studies have reported that nitrite produced using APP can function like sodium nitrite in food, especially meat products, without the risk of mutagenicity. (Kim et al., 2016; Yong et al., 2018b).

1.3. APP for meat curing

1.3.1. Direct APP treatment on meat

The application of nitrite produced by APP to meat curing mainly consists of a direct APP treatment on meat and an indirect method of adding APP treated material to meat products. Direct APP treatment involves the step of infusing plasma gas into meat in a closed chamber for interaction between plasma radicals and liquid in meat.

According to Jung et al. (2017b), as a result of direct exposure of plasma gas to meat batter using a DBD plasma system, the nitrite level of batter gradually increased over the treatment time ($P < 0.05$). As APP treatment time increased, it was observed that a^* values of cooked meat products made from the treated meat batter also increased (Lee et al., 2018b). Both experiments used the same direct APP treatment system, and the temperature of the meat batter rose depending on the treatment time due to APP gas with relatively high temperatures. In general, meat products are avoided from being exposed to high temperatures during processing due to the risk of lipid oxidation and microbial growth (Muzzalupo, 2013). Therefore, the problem of temperature rise during APP treatment on meat for curing can be a challenging issue (Jung et al., 2017b).

Jo et al. (2018) demonstrated that the heat loss of APP gas can be induced while moving through the supply pipe connected to the chamber. Although that system was operated with a high-power condition that could generate more heat and radicals, it was possible to maintain a level of heat rise similar to that of a low power system by increasing the chance for heat loss due to movement through supply pipe. These previous studies have shown that direct APP treatment of meat may be an effective method of meat curing to replace the use of sodium nitrite despite the limitation of temperature rise depending on the treatment time.

1.3.2. Use of APP-treated materials

Many studies have been conducted on APP treatment for non-meat materials such as water, plants, and plant extracts to produce nitrite-enriched natural sources. Similar to the principle of direct APP treatment on meat, nitric oxides are produced by the interaction between APP and moisture of treated materials, then decomposed through the reaction with various radicals to generate nitrite.

Jung et al. (2015b) reported that the use of APP-treated water, which increased nitrite by treating with APP to DDW containing 1% sodium pyrophosphate, could have a curing effect on sausage batter. The emulsion sausages prepared with APP-treated water showed a similar level of instrumental color, lipid oxidation, and sensory properties to sausages with sodium nitrite. Yong et al. (2017b) also reported that pork loin hams with APP-treated water had higher a^* value than ham containing sodium nitrite, which may result from the high level of nitroso-heme pigment in ham with APP-treated water.

Furthermore, an approach has been proposed to produce nitrite sources by APP treatment on natural substances such as *Perilla* plants that do not contain nitrates but have excellent antibacterial and antioxidant properties. APP treatment was found to increase the nitrite content of 70% ethanolic extract of *Perilla frutescens* L. from 0 ppm to about 46 ppm and to increase the inhibitory effect on *Salmonella* Typhimurium and *Clostridium perfringens* without compromising antioxidant activity (Jung et al., 2017a). However, it is reported that the nitrite sources made from APP-treated red *perilla* juice may be accompanied by the degradation of chlorophyll and anthocyanin in *perilla*, leading to a decrease in meat value and discoloration, thereby further studies are needed. (Lee et al., 2018c; Lee et al., 2019b).

1.3.3. Limitation of APP

APP continuously releases hydrogen ions that decrease pH during treatment (Jung et al., 2017a). However, in acidic conditions (below pH 5.5), nitrite can be decomposed to form nitric oxides, resulting in a reduction of the total nitrite level (Fox & Nicholas, 1974). For these reasons, previous studies have operated APP system with synthetic additives such as sodium hydroxide or sodium pyrophosphate to increase the initial pH, or by using certain plants, such as winter mushrooms with high natural pH values around 9 (Jo et al., 2020). However, the use of chemicals in the development of natural nitrite sources to replace synthetic sodium nitrite may be counter to the original purpose. In other words, to apply APP technology to meat curing on an industrial scale in the future, it is necessary to solve the limitation of continuous pH reduction during treatment without the use of chemicals. By overcoming the limitations of this APP, the range of materials for nitrite production might be extended to various vegetables or fruits with good flavor, high bioactive substance content, and high consumer preference, ultimately contributing to the production of high-quality meat products containing natural nitrite sources.

Chapter II.

Enriching onion powder with natural nitrite for meat curing using atmospheric pressure plasma and egg whites

This manuscript consists of part of a paper submitted to LWT- Food Science Technology as partial fulfillment of the Master's program of Ji Won Kim.

2.1. Introduction

Sodium nitrite is widely used in the meat industry as it allows meat products to preserve the unique flavor while maintaining a heat-stable red color, suppressing lipid oxidation, and extending their shelf life (Alahakoon et al., 2015). In addition, sodium nitrite has been reported as the only substance inactivating *Clostridium botulinum* which secrets lethal paralytic neurotoxins (Reddy et al., 1983), and various foodborne pathogens including *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens* (Buchanan & Solberg, 1979; Hansen & Levin, 1975; Riha & Solberg, 1975). Despite the various benefits of its use, many consumers have concerns about the effect of synthetic additives on their health. Therefore, many studies have been conducted on a replacement for synthetic sodium nitrite in the production of processed meat products (Alahakoon et al., 2015).

One of the most popular strategies to replace sodium nitrites in food production has been the addition of nitrate-enriched vegetables, which contain naturally high concentrations of nitrate, like celery, spinach, and red beet, then to have this nitrate converted to nitrite by microorganism processing. However, this approach has three important limitations: (1) this strategy can generate off-flavor originated from certain vegetables used, (2) this scheme increases the overall maintenance costs of the system (Riyad et al., 2018), and most importantly, (3) if there is not enough nitrate content originally, the kinds of vegetables to be used are limited even though they possess good flavor and various beneficial bioactivities.

Recently, APP has been evaluated as an alternative source of nitrite production without the addition of a synthetic additive (Lee et al., 2017; Lee et al., 2018c; Yong et al., 2015b). APP is a process that generates ionized gas containing RNS. When these RNS dissolve in adjacent water, they form nitrates or nitrites. A series of studies have been conducted using these properties of APP treatment to naturally increase nitrite content in water, plant extract, and meat batter. (Jung et al., 2015a; Jung et al., 2017a; Jung et al., 2017b).

If the range of APP-applicable vegetables extends to vegetables that are generally perceived as flavorful and natural from consumers, the scope of application of this technology in the commercial sphere can be much wider (Mota et al., 2010). Onions (*Allium cepa* L.) are an ideal target for this type of study as they are recognized as healthy by consumers and have been used to improve the taste of meat products in many other countries (Tang & Cronin, 2007).

APP treatment releases protons which reduces the pH (Hu et al., 2001). Under acidic conditions (below pH 5.5) nitric oxide is formed by the self-decomposition of the nitrite ion, which can lead to a decrease in total nitrite concentration (Fox & Nicholas, 1974). Therefore, previous studies that focused on nitrite production by APP used additional substances, including sodium hydroxide or sodium pyrophosphate, to increase the initial pH (Lee et al., 2018c; Jung et al., 2015b). Those studies showed that it was possible to increase nitrite concentration using APP as long as the environmental pH was properly controlled using synthetic chemicals. However, the use of chemicals in the process to replace chemical additives may be against the ultimate purpose. From these results, we hypothesized that the addition of natural materials with high pH and buffering capacity such as egg whites could be pH adjusters for APP treatment instead of chemical pH adjuster (Benton & Brake, 1996). In addition, egg white also has the advantage that its use may not compromise the quality of final products because egg white is used as a common ingredient of processed meats. Therefore, the purpose of this study was to investigate the effect of egg white as an enhancer for APP-mediated nitrite production in onions and to investigate the characteristics of APP-treated onions processed in powder form, and to apply APP-treated onion powder to the preparation of meat products to confirm that it can replace sodium nitrite.

2.2. Materials and methods

2.2.1. Experiment 1: Effect of APP treatment on fresh onions in the presence of egg whites

2.2.1.1. Sample preparation

The effect of egg white addition on nitrite production in onion by APP was analyzed in experiment 1 (Figure 1). Fresh onions obtained from a local market (Seoul, Korea) were peeled and ground using a mixer. The ground onions were divided into three groups: O, PO, and POE. For POE, egg whites were filtered using a stainless-steel sieve with a mesh size of 0.8 mm and added to the ground onion at a final concentration of 30% (w/w), which was adjusted following preliminary analyses. For the APP treatment, we used a DBD plasma system (550 W and 25 kHz; Plasmapp PCS-20 N, Plasmapp Co., Daejeon, Korea; Figure 2). Each PO and POE were APP treated in the main chamber until their pH reached 5.5, where nitrite is known to begin to convert to nitric oxide (Jung et al., 2015a).

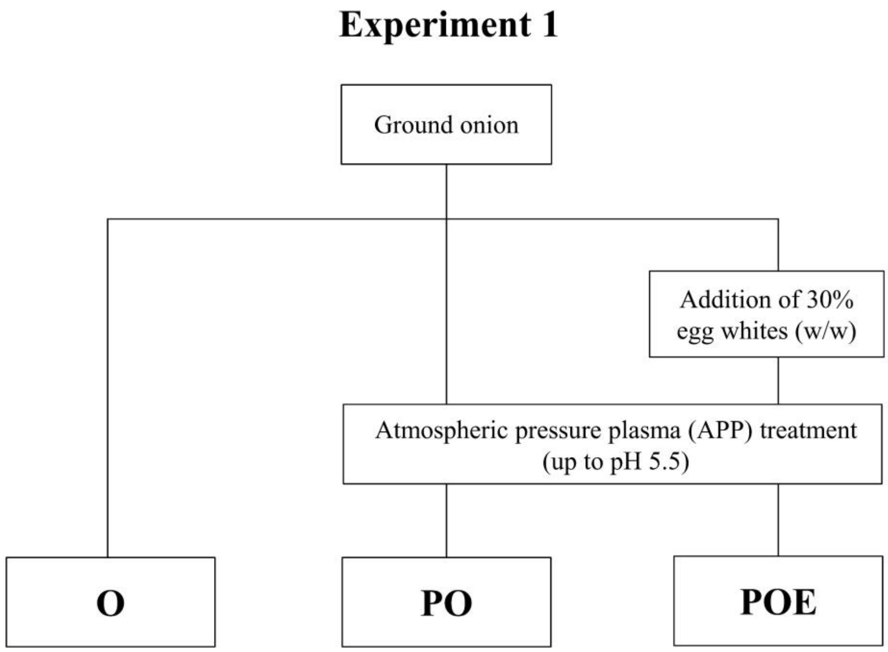

Figure 1. Design of experiment 1 with APP-treated onions in the presence of egg whites.

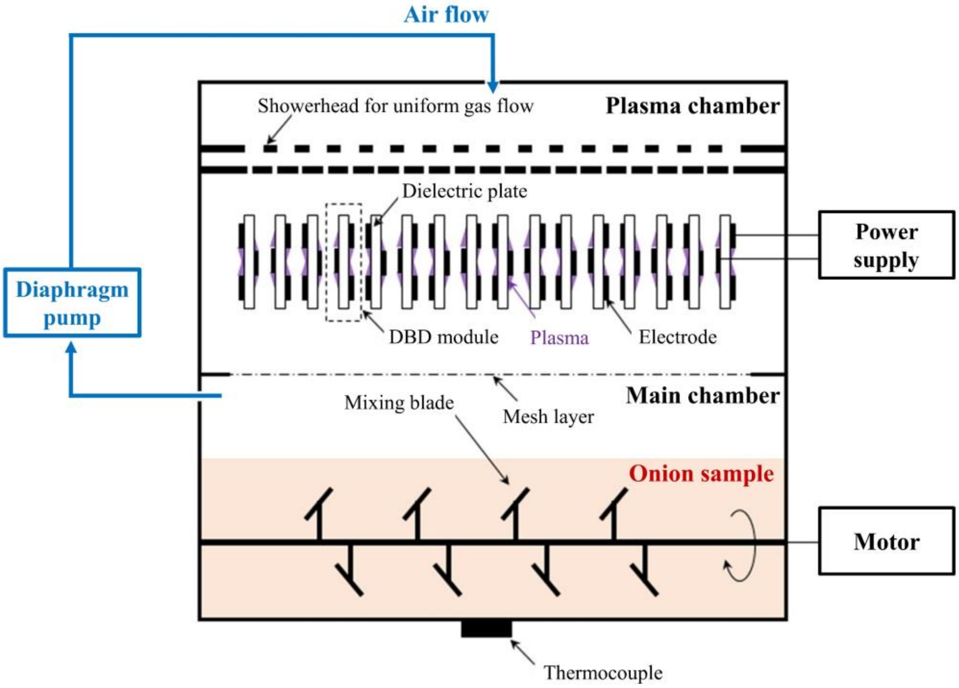

Figure 2. Schematic diagram of the experimental setup for APP treatment (adapted from Jung et al., 2017a).

2.2.1.2. pH

pH of onions during APP treatment was analyzed as follows. Onions were collected every 10 min, and APP treatment was paused each time for safe collection. One gram of sample was homogenized with 9 mL of DDW using a homogenizer (T25 digital ULTRA-TURRAX®, Ika Co., Staufen, Germany) for 20s. The homogenates were centrifuged at 2,265 × g for 10 min and filtered using filter paper (No. 4, Whatman PLC., Kent, UK). The pH of the filtered solution was measured using a pH meter (Seven 2Go, Mettler-Toledo Inc., Schwerzenbach, Switzerland), which was calibrated using standardized buffers for pH 4.0, 7.0, and 9.1 at room temperature.

2.2.1.3. Residual nitrite content

The residual nitrite content in onions during APP treatment was measured using the colorimetric method (Jung et al., 2017b). Onions were collected every 10 min in the same way as pH samples, then stored at −80°C until analysis. A total of 1 g from each sample was resuspended in 30 mL of DDW and heated in an 80°C water bath (WB-22, Daihan Scientific, Wonju, Korea) for 20 min. To prepare the test solution, each suspension was filtered into a 50-mL volumetric flask using filter paper (No. 4, Whatman PLC, Buckinghamshire, UK) and diluted to the calibration line with DDW. Then, the sample solution (10 mL) was transferred to another 50-mL volumetric flask and reacted with sulfanilamide solution and N-(naphthyl)-ethylenediamine dihydrochloride. The absorbance of the sample was measured at 540 nm. The residual nitrite content was calculated using the standard curve obtained with sodium nitrite expressed as mg kg^{-1}.

2.2.2. Experiment 2: Characteristics of APP-treated onion powder

2.2.2.1. Sample preparation

In experiment 2, all of the onion samples after the APP treatment were freeze-dried and ground into powder as shown in Figure 3 to increase applicability and storage stability. Each onion powder was stored at −20°C and vacuum-packaged in polyethylene bags until use. Then, the overall characteristics of the onion powders including residual nitrite content, proximate composition, and mutagenicity were investigated.

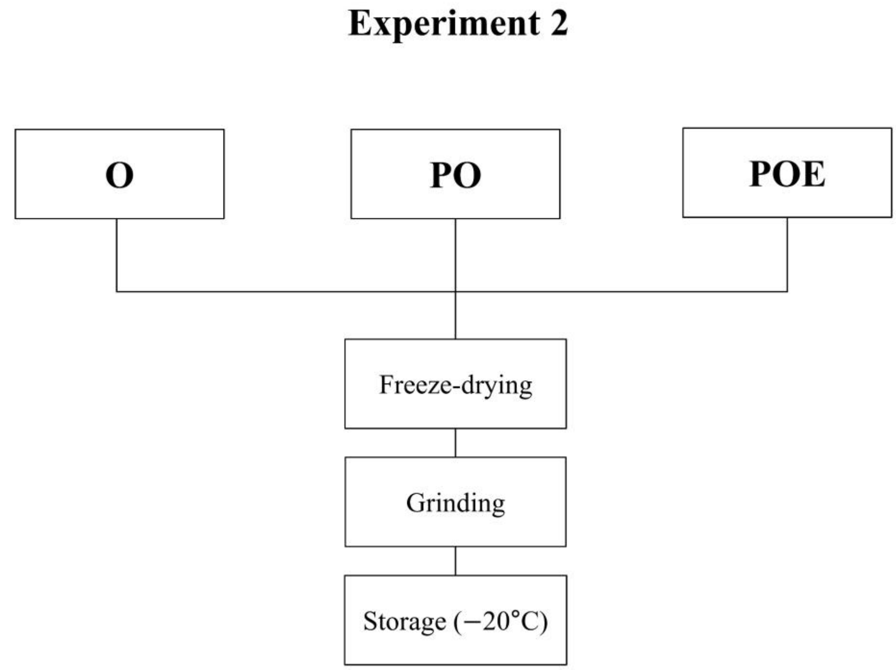

Figure 3. Design of experiment 2 with different onion powders.

2.2.2.2. Residual nitrite content

The residual nitrite content in onion powders was examined in the same way as in 2.2.1.3. and expressed in units of mg kg^{-1}.

2.2.2.3. Proximate composition

The proximate composition of onion was determined using the AOAC method. Crude protein content was measured using the Kjeldahl method (VAPO45, Gerhardt Ltd., Idar-Oberstein, Germany). Crude ash content was measured by burning 1 g of sample in a furnace at 600°C overnight and determining from the weight of the crucible before and after burning.

2.2.2.4. Instrumental color

The instrumental color of onion powder was measured using a colorimeter (CR-5, Konica Minolta Sensing Inc., Osaka, Japan). Each powder was transferred to a 35 × 10 mm plastic Petri dish then measured. The instrument was set to reflectance with Ø 30 mm aperture and calibrated using a standard black and white calibration plate (CM-A210, Konica Minolta Sensing Inc.) before analysis. The color values were expressed as CIE L^*, a^*, and b^*.

2.2.2.5. Mutagenicity

Mutagenicity assays on the onion powders were conducted using the modified Ames test (Maron & Ames, 1983). Each group of APP-treated onion powders was extracted by dissolving in 70% ethanol using a 1:20 ratio. The powder solution was stirred continuously for 24 h at room temperature in the dark. The solution was filtered using Whatman filter paper (No. 4, Whatman PLC). The ethanol remaining in the filtrate was distilled using a vacuum evaporator (Rotary Vacuum Evaporator N-11 Eyela; Tokyo Rikakikai Co., Ltd, Tokyo, Japan), and the extracts were freeze-dried (Freeze-dry system, FreeZone 18; Labconco Corp., Kansas City, MO, USA). The dried extracts were stored at −80°C until use. To establish a dose-response relationship, the extracts were diluted to three different concentrations of 313, 1250, and 5,000 μg per plate using 70% ethanol. Histidine-dependent (His^{-}) *Salmonella* Typhimurium strains, TA98 and TA100, were used to evaluate various mutagenic potential. Each strain was incubated in Oxoid nutrient broth No. 2 (Oxoid Ltd., Basingstoke, UK) and cultured for 17 h at 37°C. S9 was used to induce metabolic activation (+S9) and DDW was used for the control (−S9). With no metabolic activation, 4-NQO and SA acted as the positive control for TA 98 and TA 100 respectively. Under metabolic activation conditions, 2-AA was used as a positive control for both strains. The solvent for the positive controls was dimethyl sulfoxide, except for SA which used DDW. The negative control was 70% ethanol, the solvent used to produce the extracts. To carry out the assay, the sterilized sample solution and bacterial suspension were mixed in a sterile tube filled with top agar. S9 or DDW was also added to make metabolic activation different. The mixture was poured onto minimal glucose plates with a trace amount of histidine to allow only bacteria that revert to histidine independence (His^+) to form colonies. Each sample solution was assayed in triplicate.

2.2.3. Experiment 3: Application of APP-treated onion powder as a natural nitrite source to sausages

2.2.3.1. Sausages manufacture

In experiment 3, we manufactured sausages using the APP-treated onion powders and evaluated them for various quality criteria (Figure 4). To manufacture the sausages, fresh pork legs, backfat, and salt were purchased from a local market (Seoul, Korea) and sodium nitrite was prepared separately. The sausage batter was then separated into four groups: (1) None, (2) SN, (3) PO, and (4) POE. To create positive control, 150 ppm of sodium nitrite was added to allow 100 ppm of nitrite ions to be included in the SN sausage batter. For POE sausages, a designated amount of POE powder was added to match the nitrite content of the positive control, and PO powder was added in the same amount as POE powder. The amount of POE powder which contains 100 ppm nitrite content was determined by a preliminary examination. Meanwhile, for the negative control (None), no nitrite source was added to the sausage batter. After manufacture, all the sausages were vacuum-packaged in polyethylene bags, cooked in a water bath until the core temperature reached 72°C, then stored for 14 days in a refrigerator at 4°C.

Experiment 3

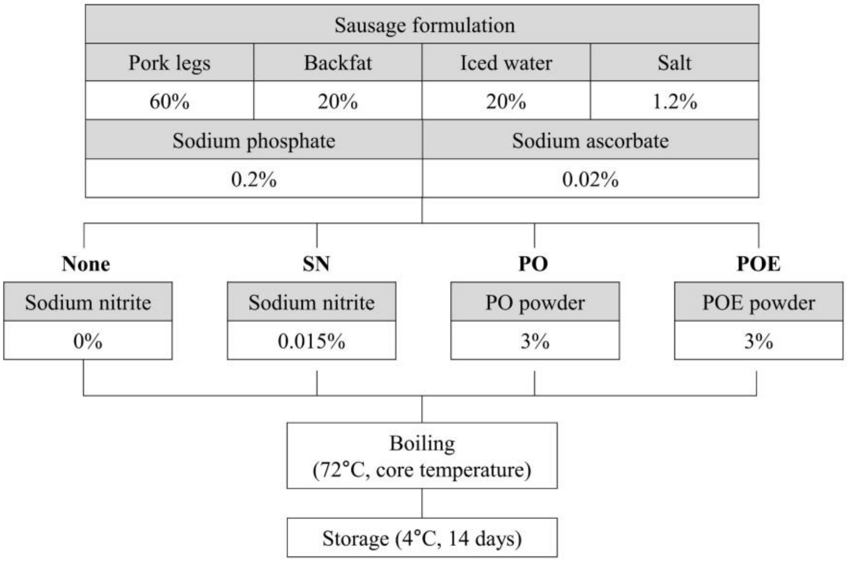

Figure 4. Design of experiment 3 for emulsion sausages with different materials.

2.2.3.2. pH

Changes in pH of emulsion sausages with different materials during 14 days of storage at 4°C was examined in the same way as in 2.2.1.2.

2.2.3.3. Residual nitrite content

Changes in residual nitrite content in sausages during storage of 14 days was examined in the same way as in 2.2.1.3. and expressed in units of mg kg^{-1}.

2.2.3.4. Instrumental color

The instrumental color of the sausages was evaluated and expressed using the same colorimeter, calibration method, and expression method as in 2.2.2.4. Each sausage (Ø 2.5 cm) were cut into 1.5 cm height then measured. The instrument was set to reflectance with an Ø 8 mm aperture.

2.2.3.5. Emulsion stability

For emulsion stability, cooking loss was measured among all sausages. Sausages were vacuum-packaged in polyethylene bags and cooked in a water bath until the core temperature reached 72°C. The core temperature was monitored using a real-time mode thermometer with a probe-type thermocouple (TM-747DU, Tenmars Electronics Co., Ltd.). The heated samples were cooled at room temperature for 30 min then determined from the weight of the sausages before and after cooking as follows (Lee et al., 2015):

$$\text{Cooking loss (\%)} = \frac{\text{Weight before cooking - Weight after cooking}}{\text{Weight before cooking}} \times 100$$

2.2.3.6. Texture profile analysis

Sausage samples (Ø 2.5 cm) were cut into 2 cm height and analyzed using a TA1 texture analyzer (AMETEK Lloyd Instruments Ltd., Fareham, UK). A compression plate of Ø 70 mm was attached to the analyzer that compressed the samples twice to 60% of their original height (test speed of 2.0 mm/s, trigger force of 0.1 N). The data were analyzed using the NexygenPlus software program (AMETEK Lloyd instruments Ltd.). The hardness (Newton, N), springiness, gumminess (N), chewiness (N), and cohesiveness were recorded.

2.2.3.7. Visual and olfactory properties

The protocol used for our analysis of the visual and olfactory properties of the sausages produced in this study was approved by the Institutional Review Board of the Seoul National University (No. 1910/001–010). Evaluation of the sausages was conducted by a panel of 10 trained panelists. Each sausage (Ø 2.5 cm) were sliced into 2 cm height and placed in different vacuum bags, to avoid mixing the flavors of different sausages during cooking. The sausages were reheated using a water bath until the core temperature reached 72°C and served to the sensory panel individually. The scoring of each sausage was done on a single sheet using a 9-point hedonic scale. The visual properties evaluated included redness (1=extremely pale, 9=extremely red), glossiness (1=slightly glossy, 9=extremely glossy) and firmness (1=extremely soft, 9=extremely hard). The olfactory properties evaluated included warmed-over flavor, egg odor, and onion odor (1=extremely weak, 9=extremely strong).

2.2.4. Statistical analysis

One-way analysis of variance (ANOVA) was performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Significance differences between the mean values were determined by Tukey's multiple range test with a confidence level of $P < 0.05$. The obtained results were reported as mean values and standard error of the mean.

2.3. Results and discussion

2.3.1. Experiment 1: Effect of APP treatment on fresh onions in the presence of egg whites

2.3.1.1. pH and hydrogen ion concentration

Prior to freeze-drying, changes in the pH of PO and POE samples during the APP treatment were measured. Before the APP treatment, the initial pH of PO and POE were 6.11 and 7.10, respectively (Figure 5a). POE had a higher pH than PO which may result from the addition of the egg whites with a high pH that ranges from pH 7.6 to 9.2 (Brown, 2011). PO and POE were then treated with APP until they reached a pH of 5.5. This cutoff point was set to avoid the decomposition of nitrite to nitric oxide under acidic conditions. When the pH acidified below 5.5, unstable nitrite ions can convert to nitrous acid (pKa = 2.8 − 3.2) and subsequently to nitric oxide via the following reactions (Bender & Schwarz, 2018; Jung et al., 2017a):

$$NO_2^- + H^+ \rightleftharpoons HNO_2$$

$$2HNO_2 \rightarrow H_2O + N_2O_3$$

$$N_2O_3 \rightarrow NO + NO_2$$

Continuous pH decline was observed in both PO and POE samples throughout the APP treatment. This phenomenon was consistent with the fact that RNS induced by APP can decrease pH by interacting with onions which have a moisture content of approximately 87% (Abdou Bouba et al., 2018). When the nitrogen in the air was discharged in the presence of water or material with high moisture contents, the pH of the treated material is known to decrease because RNS can dissolve in, producing protons through the following reactions (Jung et al., 2015a):

$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$

$N_2O_4 + H_2O \rightarrow HNO_2 + HNO_3$

$N_2O_3 + H_2O \rightarrow 2HNO_2$

$HNO_2 \rightleftharpoons H^+ + NO_2^-$

$HNO_2 \rightleftharpoons H^+ + NO_3^-$

The total APP treatment time for PO and POE was 20 and 83.3 min, respectively (data not shown). This extended treatment time in POE can be attributed to the initial pH rise and the buffering effect caused by egg whites (Benton & Brake, 1996). Proteins in egg whites such as ovalbumin can show buffering effects by involving aspartate and glutamate and terminal-carboxyl groups in their sidechains (Luo et al., 2018). The changes in the hydrogen ion concentration calculated from the pH measurement data also demonstrated the buffering effect of egg white (Figure 5b). In addition, hydrogen ion of PO was increased at a constant rate with the treatment time (R^2 = 0.9999), while that of POE was gradually increased (R^2 = 0.8521) due to the buffering effect of the added egg whites.

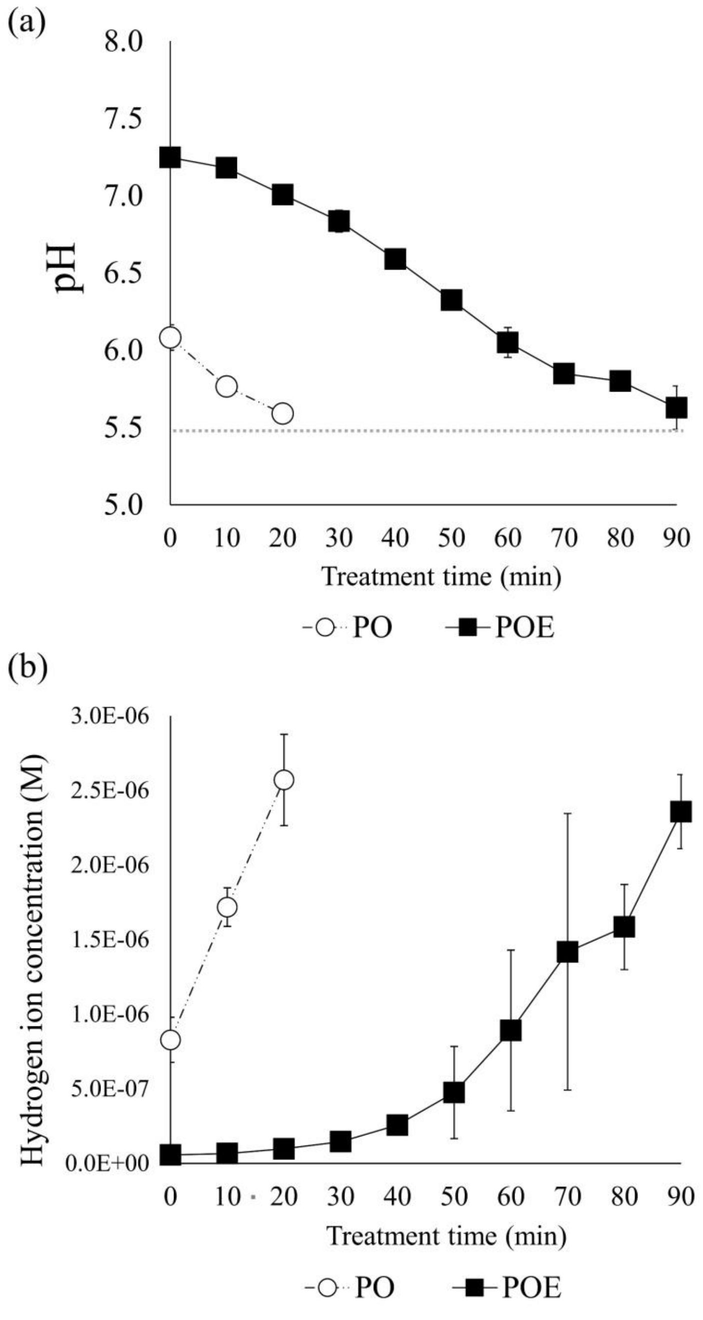

Figure 5. Changes in (a) pH and (b) hydrogen ion concentration (M) of onions (PO) and onions in the presence of egg whites (POE) during APP treatment. Error bars indicate standard deviation.

2.3.1.2. Residual nitrite content

Residual nitrite content in POE was higher than PO, as nitrite production continued during the treatment time extended (Figure 6). Choe et al. (2016) reported that the pKa value of nitrous acid is 3.37, which means that 50% of the nitrite ion dissociates at pH 3.37. Braida & Ong (2000) also noted that when the pH is higher than 5.3, 99% of total nitrites exist as nitrite ion and only 1% is present as nitrous acid. For these reasons, the present study was designed to complete the APP treatment before the pH of onions was lowered below 5.5, and it was confirmed that the pH of POE slowly decreased compared to PO until the final pH was reached. As a result, nitrite generated from RNS could be accumulated for a relatively long time in POE. Although the nitrite content of the fresh onions was not detectable, the final nitrite contents of PO and POE increased to 110.88 and 403.74 mg kg^{-1} after APP treatment, respectively. Consequently, significantly higher nitrite content in POE among all onion samples indicated that the addition of egg white during APP treatment could improve the efficiency of nitrite production.

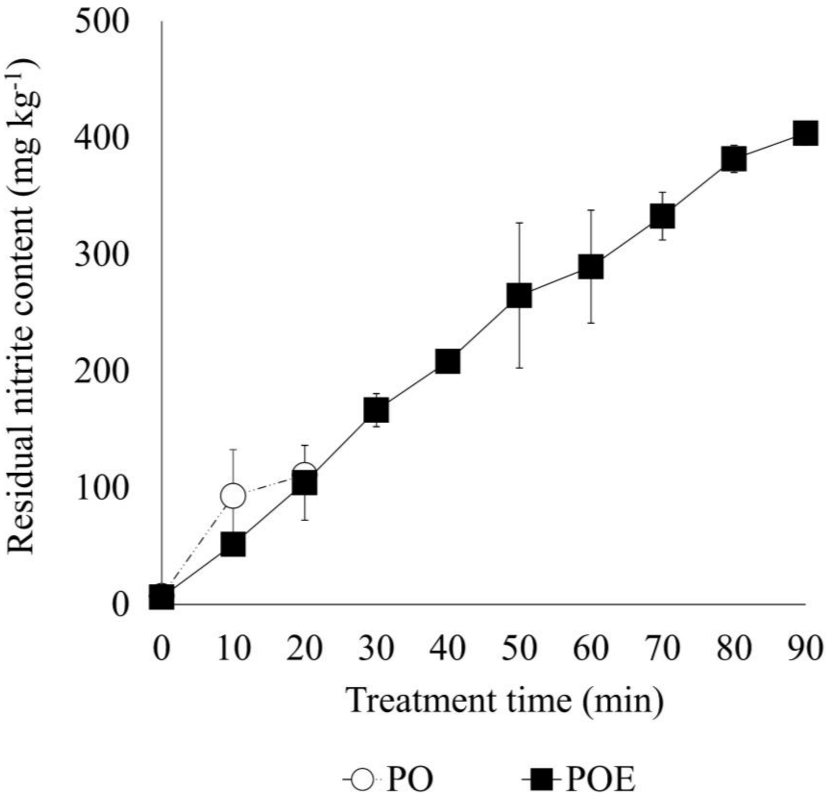

Figure 6. Changes in residual nitrite content (mg kg^{-1}) of onions (PO) and onions in the presence of egg whites (POE) during APP treatment. Error bars indicate standard deviation.

2.3.2. Experiment 2: Characteristics of APP-treated onion powder

2.3.2.1. Residual nitrite content

Similar to the experiment 1, POE powder resulted in the significantly higher residual nitrite content than PO powder, while O powder had nitrite content close to the detection limit because the nitrite content of the onion is originally low enough to be undetectable (Shokrzadeh et al., 2008). The residual nitrite content of PO and POE powder was generally more concentrated in powder form than in mixtures form due to the evaporation of moisture during freeze-drying. POE powder had a significantly concentrated nitrite content of 3226.72 mg kg^{-1}, which was even 402.8 times higher than O powder containing about 8.01 mg kg^{-1}. In PO powder, the nitrite content was also increased to 508.88 mg kg^{-1}, about 63.5 times higher than that of O powder. POE, which had a longer APP treatment time than PO, had the highest nitrite content due to the added egg whites with high pH and buffering capacity. So far, vegetables which have high nitrate content were usually applied as nitrite substitutes. However, these results indicate that, regardless of original nitrate content, APP can induce the production of nitrite with any materials especially when egg whites are added. Also, APP-treated onion mixtures can be processed in a powder form for industrial use without excessive loss of produced nitrite.

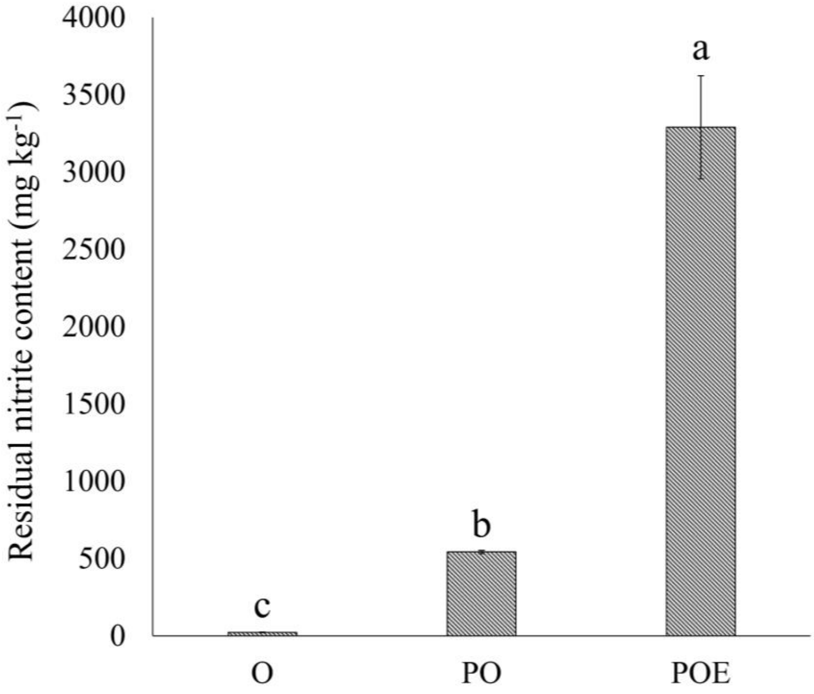

Figure 7. Difference in residual nitrite content (mg kg^{-1}) between different onion powders. Powder samples were made from fresh onion (O); APP-treated onion (PO); APP-treated onion in the presence of egg whites (POE). Error bars indicate standard deviation. $^{a\text{-}c}$Different letters indicate significant differences ($P < 0.05$).

2.3.2.2. Proximate composition

The proximate composition was analyzed to determine the nutritional value of APP-treated onion powders (Table 1). There were no differences in the crude protein and ash contents between O and PO powders. Based on this result, we suggest that APP treatment did not affect the composition of the onion powders. However, the addition of egg white resulted in an increase in both crude protein and ash content for the POE powder ($P < 0.05$). Egg white is known to contain 90% water, 10% protein, and a trace amount of ash content (McGee, 2007). Therefore, the addition of egg whites may increase the crude protein and ash content of the POE powder, which may result in improved nutritional value when this powder added to protein foods such as meat products.

Table 1. Proximate composition (%) of different onion powders

Proximate composition	Treatment[1]			SEM[2]
	O	PO	POE	
Crude protein (%)	11.26[b]	7.23[b]	28.93[a]	1.002
Crude ash (%)	4.10[b]	4.09[b]	4.75[a]	0.981

[1]Powder samples made from fresh onion (O), APP-treated onion (PO), APP-treated onion in the presence of egg whites (POE).
[2]Standard error of the means (n = 9).
[a, b]Different letters within the same row indicate significant differences ($P < 0.05$).

2.3.2.3. Instrumental color

Instrumental color of onion powders was analyzed because the color of the additive can affect the color of the final product (King & Whyte, 2006). The L^* values were highest in the O powder followed by the PO and POE powders ($P < 0.05$, Table 2). In both a^* and b^* values, POE powder was shown to be the highest with O recording the lowest values ($P < 0.05$). When compared to O powder, made from fresh onions, both PO and POE powders showed lower L^* values and higher a^* and b^* values ($P < 0.05$). The color difference of each powder is also confirmed in Figure 8. There were also differences in color between the POE and PO powders, which may be attributed to the inherently high a^* and b^* value of egg whites when dried (Chen et al., 2012).

Table 2. Instrumental color values of different onion powders

Instrumental color	Treatment[1]			SEM[2]
	O	PO	POE	
L^* value	89.18[a]	81.18[b]	78.17[c]	0.604
a^* value	−2.28[c]	1.81[b]	3.54[a]	0.369
b^* value	15.64[b]	19.99[ab]	24.38[a]	1.049

[1]Powder samples made from fresh onion (O), APP-treated onion (PO), APP-treated onion in the presence of egg whites (POE).
[2]Standard error of the means (n = 9).
[a-c]Different letters within the same row indicate significant differences ($P < 0.05$).

(a) **(b)** **(c)**

Figure 8. The appearance of different onion powders. Powder samples were made from (a) fresh onion (O); (b) APP-treated onion (PO); (c) APP-treated onion in the presence of egg whites (POE).

2.3.2.4. Mutagenicity

The Ames test is widely used to detect the carcinogenic potential of chemicals which may result in gene mutations (Oliveira et al., 2007). A product is considered to have high mutagenic potential if the number of His^+ revertant colonies is higher than in the negative control or if dose-related increases in the number of His^+ revertant are observed (Kim et al., 2016). In this study, the Ames test was used to evaluate the mutagenic potential of PO and POE powders (Table 3). The number of revertant colonies, from His^- to His^+, was similar between the PO and POE powders and the negative control regardless of the strain or the presence of S9 mix. There was no dose-dependent increase in either PO or POE powder samples and no difference in the number of His^+ revertant colonies was found between the PO and POE powders. Based on these results, APP-treated onion powders can be considered to be safe for application in food processing as they did not show mutagenic potential. Kim et al. also reported that there was no mutagenicity or immune toxicity in emulsion sausages manufactured using APP-treated water.

Table 3. Number of revertant colonies in a *Salmonella* Typhimurium reversion assay done on the extract from different onion powder

Treatment[1]	Dose (μg/plate)	Number of revertant colonies (His^+) per plate			
		TA98 (−S9)	TA98 (+S9)	TA100 (−S9)	TA100 (+S9)
PO	313	79[2] ± 40	135 ± 78	553 ± 107	406 ± 21
	1250	48 ± 5	104 ± 15	322 ± 47	411 ± 36
	5000	36 ± 10	115 ± 44	441 ± 148	293 ± 50
POE	313	55 ± 8	48 ± 17	301 ± 93	389 ± 185
	1250	50 ± 27	52 ± 23	265 ± 52	344 ± 72
	5000	47 ± 12	69 ± 15	194 ± 75	263 ± 31
Negative control	70% Ethanol	39 ± 12	92 ± 19	396 ± 30	421 ± 82
Positive control	4-NQO	356 ± 145			
	2-AA		1559 ± 558		
	SA			1058 ± 424	
	2-AA				1691 ± 127

[1]Extract from the onion powder made from APP-treated onion (PO) and APP-treated onion in the presence of egg whites (POE).
[2]Means ± standard deviation (n = 3).

2.3.3. Experiment 3: Application of APP-treated onion powder to sausage processing

2.3.3.1. pH

The initial pH of all sausages on the day of manufacture was not significantly different, indicating that APP-treated onion powders did not affect the pH of sausages (Table 4). When a substance or solution is directly exposed to APP, pH reduction can occur by the reaction between acidic ions produced from RNS and water molecules (Kim et al., 2013 However, in this study, PO and POE sausages were free from this strong pH reduction since APP treatment was not performed on the sausage itself but one of the ingredients. Also, pH among different sausage groups on the same day did not significantly differ during all storage days ($P < 0.05$). Meanwhile, None and POE sausages showed a slight decrease in pH during 14 days. The pH reduction in meat products during storage can be caused by the fermentation of carbohydrates by microorganisms to produce lactic acid (Fista et al., 2004).

Table 4. Changes in pH of emulsion sausages with different materials during 14 days of storage at 4°C

Treatment[1]	Storage (day)			SEM[2]
	0	7	14	
None	6.17[a]	6.11[b]	6.12[b]	0.006
SN	6.18	6.13	6.12	0.026
PO	6.14	6.07	5.99	0.063
POE	6.16[a]	6.10[a]	5.81[b]	0.059
SEM[3]	0.014	0.015	0.076	

[1]Sausage enriched with no nitrite source (None); sodium nitrite (SN); APP-treated onion powder (PO); APP-treated onions powder in the presence of egg whites (POE).
[2]Standard errors of the means (n=9), [3](n=12).
[a, b]Different letters within the same row indicate significant differences ($P < 0.05$).

2.3.3.2. Residual nitrite content

The residual nitrite content of the None, SN, PO, and POE sausages on 0 day was 7.80, 83.77, 35.70, and 77.10 mg kg^{-1}, respectively (Figure 9). Both POE and SN sausages showed a high nitrite content, with a slightly higher nitrate content in POE (P < 0.05). PO sausage had a significantly lower nitrite content because nitrite production was relatively low compared to POE. All of the sausages showed a significant decrease in the residual nitrite content, except for the SN sausages (P < 0.05) during storage. In the case of the None sausages, the initial nitrite content was close to the detection limit and it continuously decreased over the storage days. Both PO and POE sausages showed a significant decrease in nitrite over the storage days regardless of egg white addition (P < 0.05). Many plants including onions are known to contain various antioxidants, including vitamin C, ferulic acid, and gallic acid, which can scavenge nitrite (Lee et al., 2017; Prakash et al., 2007). Therefore, the reaction of the nitrites with the natural antioxidants in the onions may result in the continuous reduction in residual nitrite content in PO and POE sausages during storage.

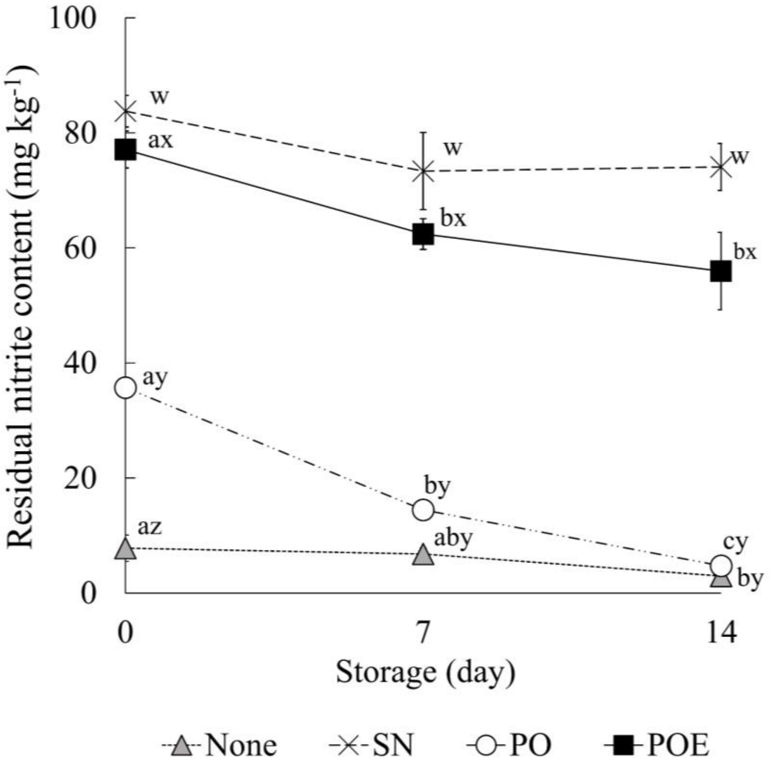

Figure 9. Changes in residual nitrite content (mg kg^{-1}) in emulsion sausages with different materials during 14 days of storage at 4°C. Sausages were enriched with no nitrite source (None), sodium nitrite (SN), APP-treated onion powder (PO), and APP-treated onion powder in the presence of egg whites (POE). Error bars indicate standard deviation. $^{a\text{-}c}$Different letters indicate significant differences between different storage days in the same sausage (P < 0.05). $^{w\text{-}z}$Different letters indicate significant differences between different sausages on the same storage day (P < 0.05).

2.3.3.3. Instrumental color

Changes in the instrumental color of sausages stored at 4°C during 14 days are shown in Table 5. During all storage days, no difference in L^* value was observed regardless of the addition of different nitrite sources. In addition, there was no change in the L^* value of all sausages with increasing the storage day. The lowest b^* value was observed in SN sausage on day 0 ($P < 0.05$), however, there was no significant difference in b^* value among all sausages on day 7. On day 14, SN sausages showed again a lower b^* value than that of PO and POE sausages. In a^* value, only None sausage showed significant changes during storage days, whereas the other sausages maintained similar a^* values. This indicates that the vivid red color of SN and POE sausages could be stable during storage days. Although POE and PO powder had higher b^* value, sausages containing those powders showed higher a^* values than that of None sausage due to the curing effect by nitrite in those powders. In particular, POE sausages showed the possibility of replacing sodium nitrite by representing higher or similar a^* value to that of SN sausages. This result was also consistent with the appearance color of the sausage cross-section (Figure 10).

Table 5. Instrumental color values of emulsion sausages with different materials during 14 days of storage at 4°C

Treatment[1]	Storage (day)			SEM[2]
	0	7	14	
L^* value				
None	68.14	68.05	68.67	0.514
SN	67.90	66.86	67.36	0.652
PO	65.47	66.85	66.73	0.678
POE	67.76	66.35	66.19	0.997
SEM[3]	0.658	0.770	0.762	
a^* value				
None	2.22[bz]	2.77[ay]	1.90[bx]	0.127
SN	6.95[x]	7.53[w]	6.81[w]	0.194
PO	4.57[y]	5.02[x]	3.77[x]	0.482
POE	7.88[w]	6.99[w]	7.15[w]	0.491
SEM[3]	0.191	0.320	0.508	
b^* value				
None	14.80[w]	14.64	14.66[wx]	0.193
SN	12.19[x]	13.74	12.86[x]	0.478
PO	15.02[w]	14.20	14.79[w]	0.455
POE	14.48[w]	14.47	15.62[w]	0.282
SEM[3]	0.236	0.440	0.407	

[1]Sausage enriched with no nitrite source (None); sodium nitrite (SN); APP-treated onion powder (PO); APP-treated onions powder in the presence of egg whites (POE).
[2]Standard errors of the means (n = 9), [3](n = 12).
[a, b]Different letters within the same row indicate significant differences ($P < 0.05$).
[w–z]Different letters within the same column indicate significant differences ($P < 0.05$).

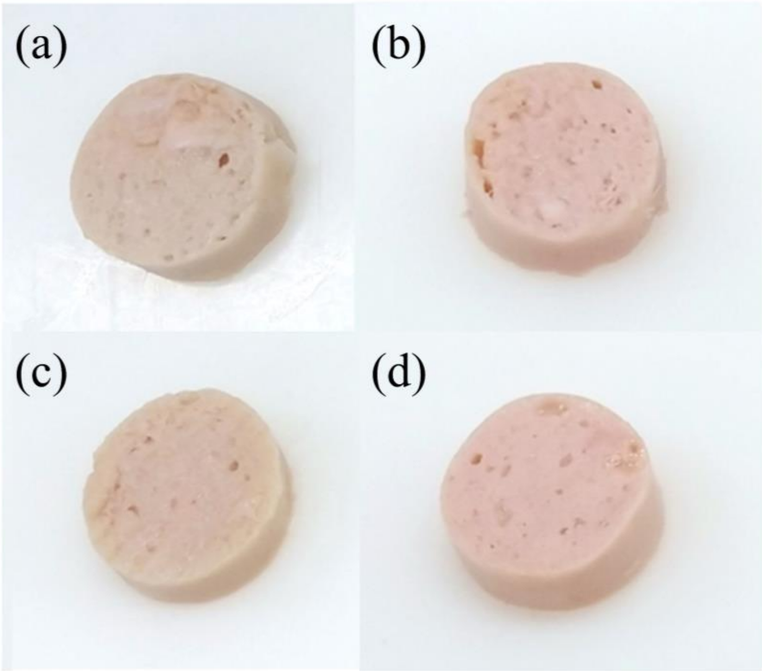

Figure 10. Cross-section of emulsion sausages with different materials. Sausages were enriched with (a) no nitrite source (None); (b) sodium nitrite (SN); (c) APP-treated onion powder (PO); (d) APP-treated onion powder in the presence of egg whites (POE).

2.3.3.4. Emulsion stability

The effect of the addition of APP-treated onion powder on the emulsion stability of sausage was analyzed using the measurement of cooking loss. The emulsion stability of meat products can be determined by the stable formation of three-dimensional networks of proteins, water, and lipids. In general, meat products with high emulsion stability have less separation of water and lipids during thermal treatment due to their stable matrix, resulting in a low cooking loss (Cofrades et al., 2000). In this study, PO sausages showed significantly lower cooking loss than None and SN sausages, meaning that it had the highest emulsion stability among all sausages (Figure 11). This high emulsion stability of PO sausage can be attributed to the high amount of dietary fiber present in onion (approximately 10.2%; Marlett & Vollendorf, 1993), similar to the other previous studies. Ham et al. (2017) reported that lotus rhizome powder had approximately 11.85% dietary fiber and its addition to sausage resulted in an increase in emulsion stability. Also, Lee et al. (2018a) found that seaweed, which is known to contain about 3.4% of dietary fiber (Kim et al., 1988), can be used in the manufacture of emulsion sausage to stabilize meat emulsions and improve texture and water holding capacity. The changes in texture properties with the addition of dietary fiber can be explained by the water-binding ability and swelling properties of the insoluble fiber (Thebaudin et al., 1997).

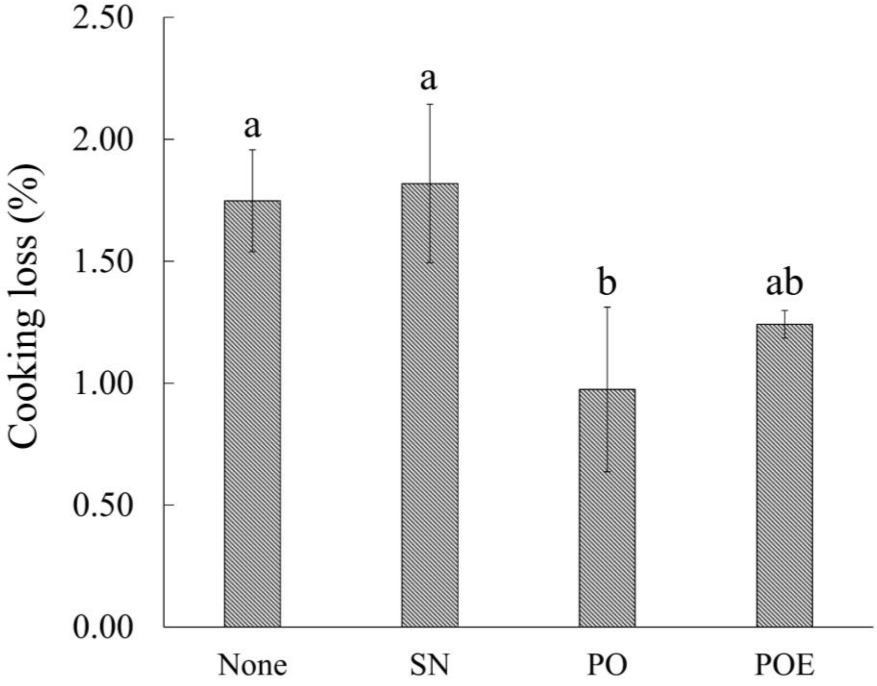

Figure 11. Cooking loss (%) of emulsion sausages with different materials. Sausages were enriched with no nitrite source (None); sodium nitrite (SN); APP-treated onion powder (PO); APP-treated onion powder in the presence of egg whites (POE). Error bars indicate standard deviation. [a, b]Different letters differ significantly ($P < 0.05$).

2.3.3.5. Texture profile analysis

The texture of sausage is formed through interaction between charged polymers, which are fragments of muscle fibers, and the texture properties of meat products are affected by water holding capacity, emulsion stability, gel formation ability, as well as adhesion between particles. (Choi et al., 2008; Shin et al., 2020). Table 6 shows the instrumental texture properties of emulsion-type sausages formulated with different materials including PO and POE powder. Both PO and POE sausage showed the greatest hardness and springiness, respectively ($P < 0.05$), whereas there was no significant difference in gumminess, chewiness, and cohesiveness value of all sausages. In particular, POE sausage contained APP-treated onion powder, which accounted for even 3% of the total composition but showed a stronger gel strength compared to None and SN sausage with relatively little or no agent. Both dietary fibers of onions and egg white protein may be responsible for this increase in the hardness of POE sausage. In an experiment by Huang et al. (2011), adding 3.5 to 7% of wheat fiber or oat fiber to Chinese sausages increased hardness. The increase in hardness was also observed in emulsion sausages added with other types of dietary fiber such as citron peel (Lee et al., 2004). Through a similar mechanism, the rich dietary fiber content in onions (approximately 10.2%; Marlett & Vollendorf, 1993) may also have a significant effect on sausage hardness. In terms of springiness, it was also reported that inulin and pectin, a type of soluble fiber, could increase the springiness of Frankfurt sausage due to water retention capacity (Méndez-Zamora et al., 2015). Meanwhile, there is still much controversy over the effect of egg white on the texture of meat emulsions. Our data were consistent with those from previous studies which reported that egg whites can increase the hardness of meat products (Carballo et al., 1996; Fischer et al., 1994). According to Carballo et al. (1996), egg whites can increase hardness by strengthening the protein network matrix in meat emulsion without affecting springiness and cohesiveness. These results suggest that the textural properties of emulsion sausage could be improved by using POE powder.

Table 6. Texture profile of emulsion sausages with different materials

Texture parameter[1]	Treatment				SEM[2]
	None	SN	PO	POE	
Hardness (N)	16.82[b]	16.93[b]	24.32[ab]	29.00[a]	2.252
Springiness	0.85[b]	0.87[b]	0.97[a]	0.89[ab]	0.864
Gumminess (N)	7.29	9.73	9.86	12.53	1.125
Chewiness (N)	6.69	8.39	9.60	11.27	1.166
Cohesiveness	0.39	0.59	0.43	0.36	0.076

[1]Sausages were enriched with no nitrite source (None); sodium nitrite (SN); APP-treated onion powder (PO); APP-treated onion powder in the presence of egg whites (POE).

[2]Standard errors of the means (n = 12).

[a, b]Different letters within the same row indicate significant differences ($P < 0.05$).

2.3.3.6. Visual and olfactory properties

Table 7 shows the results from the evaluation of the visual and olfactory properties of each of the four different sausages created in this study. For the visual properties, there were no differences between the sausages in terms of firmness, with PO sausages showing reduced glossiness compared to the others (None, SN, and POE). The score for redness and glossiness in POE sausages was similar to SN sausages while those for PO and None sausages were significantly lower. It is noteworthy that POE sausages retained similar redness scores to the SN sausages due to the increased nitrite contents in onion powder by APP treatment in the presence of egg whites. The similar redness scores for POE and SN sausages also coincided with the results of the instrumental color analysis and cross-section of the sausages.

With respect to the olfactory properties, warmed-over flavor, onion odor, and egg odor, with each evaluator asked to assess the sausages for the rancidity, the intensity of their onion odor, and specific off-flavor caused by the egg white. The warmed-over flavor of sausages is associated with lipid oxidation and can reduce consumer satisfaction and should thus be avoided (Mielche & Bertelsen, 1994). Since this warmed-over flavor can be prevented by the addition of nitrite, the intensity of this flavor can be used as an indicator of the efficacy of the sodium nitrite alternatives (Igene et al., 1985). In this study, the warmed-over flavor of the None sausages scored higher than the SN, PO, and POE sausages, which all scored similarly ($P < 0.05$). This result indicates that nitrite in the PO and POE powders may inhibit the warmed-over flavor, making them effective replacements for synthetic nitrites. Besides, Dwivedi, Vasavada, and Cornforth (2006) found that spice ingredients can reduce the warmed-over flavor in cooked beef via the masking effect. Also, other report has demonstrated that onion and its extract may cover the off-flavor of irradiated cooked meat while maintaining the natural flavor of onions (Kim et al., 2014). Therefore, PO and POE powders may inhibit the warmed-over flavor of the sausages by covering it with their onion. Both PO and POE scored higher on onion odor than None and SN sausages which could be favored by consumers because of its harmony with meat products (Janoszka, 2010). This onion odor seemed to be added to both PO and POE sausages regardless of the egg white content of the powder. In addition, the score of the egg odor in the POE sausages was not significantly different than the other sausages, even though egg whites can add to an "off-odor" of products via the browning or Maillard reaction (Mountney, 2017). Consequently, the addition of POE powder to sausages resulted in a similar effect to the synthetic nitrite additives in terms of both visual and olfactory properties as well as in residual nitrite content. Therefore, POE powder is expected to enhance the sensory quality of meat products instead of sodium nitrite by generating appropriate redness and consumer-friendly onion odor without causing warmed-over flavor and egg odor.

Table 7. Sensory properties of emulsion sausages with different materials

Sensory properties[1]	Treatment[2]				SEM[3]
	None	SN	PO	POE	
Visual properties					
Redness	3.73[b]	6.45[a]	3.63[b]	6.23[a]	0.084
Glossiness	5.07[ab]	6.20[a]	4.77[b]	5.53[ab]	0.261
Firmness	5.30	5.13	4.17	5.37	0.330
Olfactory Properties					
Warmed-over flavor	3.60[a]	2.60[ab]	2.40[b]	2.00[b]	0.224
Onion odor	1.27[c]	1.83[c]	5.81[a]	4.77[b]	0.196
Egg odor	1.83	2.27	1.93	1.97	0.267

[1]Sausages were scored on a 9-point scale; redness (1: extremely pale, 9: extremely red), gloss (1: slightly glossy, 9: extremely glossy), firmness (1: extremely soft, 9: extremely hard), warmed-over flavor, onion odor, and egg odor (1: extremely weak, 9: extremely strong).
[2]Sausage enriched with no nitrite source (None), sodium nitrite (SN), APP-treated onion powder (PO), APP-treated onion powder in the presence of egg whites (POE).
[3]Standard error of the mean (n = 12).
[a-c]Different letters within the same row indicate significant differences ($P < 0.05$).

2.4. Conclusion

The results of this study suggest that the addition of egg whites during APP treatment may increase nitrite content in onion powders without increasing the mutagenic potential and its application in the meat processing could help to improve the quality of meat products. The meat curing using APP and egg whites could allow meat producers to produce high-quality meat products with various natural nitrite sources like nitrite-enriched onion instead of synthetic sodium nitrite. The onion powder produced through this approach could improve the emulsion stability and textural properties of meat products. It could also enhance the sensory quality of meat products, expressing redness similar to sodium nitrite-added meat, adding a consumer-friendly onion odor, and reducing unpleasant warmed-over flavor. Consequently, the addition of egg whites during APP treatment on onions and the application of its powder may be an effective way to replace synthetic sodium nitrite in meat products and various processed foods.

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Summary in Korean

대기압 플라즈마와 난백을 활용한 양파 분말 내 천연 아질산 증진방안 및 육제품에의 적용

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본 연구에서는 아질산 및 질산을 거의 함유하지 않은 양파를 난백과 함께 대기압 플라즈마 처리해 천연 아질산 소재로 가공하고, 이를 실제 육가공품 제조에 적용하여 해당 소재의 아질산염 대체 가능성을 확인하고자 한다. 실험은 다음과 같이 세 단계로 나누어 진행되었다. (1) 생양파를 플라즈마 처리하여 천연 아질산 소재를 생산하는 과정에서 pH 완충효과가 있는 난백의 첨가를 통한 아질산 생성효율의 증진 여부를 확인하였고, (2) 생산한 아질산 소재의 저장성 및 산업적 활용 가능성을 확보하기 위해 분말 형태로 가공한 후 그 특성을 조사하였으며, (3) 최종적으로, 이를 실제 육가공품 염지에 적용하여 가공된 분말의 아질산염 대체 가능성을 확인하였다.

실험 1의 결과, 중량 대비 30% 수준의 난백을 첨가한 양파에서 플라즈마 처리에 의한 pH 저하가 효과적으로 지연됨에 따라 총 플라즈마 처리시

간이 증가되었고, 측정된 잔류 아질산 함량 또한 양파만 단독으로 플라즈마 처리한 것에 비해 4배 이상 높아져 난백의 첨가가 플라즈마의 아질산 생성 효율을 향상할 수 있음을 확인하였다. 실험 2에서는 산업적 활용성 증진을 위해 생산된 아질산 농축 양파 소재를 동결건조 후 분쇄하여 분말 상으로 가공하였으며, 이 과정에서 아질산은 소실되지 않고 분말 내에 높은 함량으로 잔존하는 것을 확인하였다. 또한, 플라즈마 처리한 양파 분말은 난백 첨가 여부와 상관없이 복귀 돌연변이 유발성을 나타내지 않았으며, 난백 함유 양파 분말의 조단백 및 조회분 함량은 생양파 분말 또는 플라즈마 단독처리한 양파 분말보다 유의하게 높은 것으로 나타났다. 실험 3에서는 첨가한 소재의 종류를 달리하여 유화형 소시지를 제조한 후 각각의 품질을 평가하였고, 아무것도 첨가하지 않은 소시지, 아질산염 첨가 소시지, 단독으로 플라즈마 처리한 양파 분말 및 난백과 함께 플라즈마 처리한 양파 분말 첨가 소시지의 네 그룹으로 나누어 진행하였다. 실험 결과, 난백 함유 양파 분말 첨가 소시지는 잔류 아질산 함량, 적색도 항목에서 아질산염 첨가 소시지와 유사한 수준을 나타냈으며, 유화 안정성 및 조직감 특성은 다른 소시지들에 비해 유의하게 개선되었다. 외관 및 후각 평가 결과, 난백 함유 양파 분말을 첨가한 소시지는 외관상의 적색도 항목에서 아질산염 첨가 소시지와 비슷한 점수를 기록하였으며 유의적인 차이는 없었다. 또한, 난백 함유 양파 분말을 첨가한 소시지는 소비자에게 부정적으로 인식될 수 있는 가열 산패취 항목에서 가장 낮은 점수를, 양파 향 항목에서는 유의하게 높은 점수를

획득한 것으로 나타났다. 결론적으로, 난백과 함께 플라즈마 처리한 양파 분말은 유화형 소시지에서 아질산염을 첨가하는 것과 유사한 효과를 보일 뿐 아니라, 소시지의 유화 안정성 및 풍미를 향상할 수 있어 산업에서 고품질의 육가공품 제조를 위한 천연 아질산 농축 소재로써 활용될 수 있을 것으로 사료된다.