Master's Thesis of Inseop Choi

Quality monitoring of vacuum-packed meat with olfactory receptor-embedded nanodiscs

후각 수용체 삽입 나노디스크를 이용한 진공포장육의 품질 모니터링

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Abstract

Meat is a perishable food, and monitoring of its deterioration is essential. Therefore, physicochemical assays and chemical analyses are carried out to monitor meat deterioration. On the other hand, vacuum-packing is a storage method of meat that is the most oftenly used by meat companies due to various advantages such as economic merits and flavor enhancement through wet-aging that occurs during the storage period. However, there are still no standards for the deterioration or quality of vacuum-packed meats. This is because the physicochemical and bacterial properties of vacuum-packed meat are different from packaged meat. Therefore, the quality of vacuum-packed meat is evaluated by empirical methods and sensory evaluation. To replace them, olfactory receptor-embedded nanodiscs were used to monitor the odorants from sample and pattern it so that the state of the sample could be confirmed. OR2J2, OR2W1, and TAAR5 were produced by *E. coli* and confirmed to be embedded in nanodiscs. Later, the reactivity of olfactory receptor embedded nanodiscs to odorants was confirmed through tryptophan quenching assay. Finally, the vacuum-packed meat samples with different storage durations (0, 14, 28, 42, 56 ,70 days) were treated to receptor-embedded nanodiscs. As a result, reaction pattern change of olfactory receptor embedded nanodiscs is confirmed to be related to deterioration. Thus, olfactory receptor-embedded nanodiscs are potential material for quality monitoring of vacuum-packed meat.

Keyword : Olfactory receptor, Nanodisc, Vacuum-packed meat, Meat deterioration, Food quality monitoring
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Chapter 1. Introduction

1.1. Deterioration of vacuum-packed meat and its monitoring

Meat is one of a most perishable foods [1]. If the storage duration is prolonged excessively, deterioration of meat occurs. Deterioration of meat occurs both biochemical and physicochemical ways [2]. Microbial growth and their metabolism, lipid oxidation, and enzymatic action are known as critical factors of meat deterioration. Not only off-flavor is produced during deterioration but also toxicity is. Thus, monitoring and determination of meat deterioration is essential to avoid these hazards. Physicochemical assays like pH, total aerobic bacteria (TAB), colorness, volatile basic nitrogens (VBN), and lipid oxidation test with thiobarbituric acid (TBARS assay) are frequently used to determine quality of meat products. Legal standard of meat product quality is determined based on the assays written above.

When the meat is stored for certain period time for improvement of flavor, texture and overall palatability, the meat is aged, and such process is called aging [3]. Herein, vacuum-packing of meat product can induce aging. Wet-aging is an aging process which stores vacuum-packed meat in refrigerated environment. Meat undergone wet-aging is called as wet-aged meat. It has unique flavor described as sour, ‘serumy’ (also described as bloody), and metallic flavors developed while aging period [4]. Wet-aging is economic, shelf-life extending, and easy to produce [5]. Thanks to these advantages in various areas, It' s the most dominant packaging method in meat industry.

However, there is no regulation and legal standard for vacuum-packed meat deterioration and quality control. Many factors involved in deterioration differ by meat samples [6] because of various methods used for aging of meat were modified by manufacturer and its physicochemical properties are not alike normally packed meat products which are packed under aerobic condition. Mostly, microbial properties of vacuum-packed meat differ from other meat products because of anaerobic condition of vacuum-packed meat. Consequently, deterioration of vacuum-packed meat is poorly understanded and not standardized [7].

Thus, qualifying vacuum-packed meat is carried out by sensory evaluation and empirically based methods. Due to its incorrectness and time-consuming nature, replacement of those qualifying methods has been intensively tried. Notably, chemical methods, such as GC-MS analyses [8], electronic tongue analyses [9], and polymer-based sensor analysis [10], had been tried to analyze volatile organic compounds (VOCs) produced while meat aging. They tried to quantify VOCs and to find out hit compounds or biomarkers related to meat deterioration and sensory properties. These compound-focused analyses find relations of ligands with sensory properties. Their genuine reactivities with sensory receptors are ambiguous, and their results sometimes don’t match to empirical knowledge and sensory evaluation results. Mimicry of genuine response of human sensory system are studied intensively to replace empirical methods and sensory evaluation and overcome the limitations of analysis methods.

1.2. Application of receptor-embedded nanodiscs as sensing material

G-protein coupled receptors (GPCRs) are known as receptors of chemical senses like taste, and odor [11]. Olfactory receptors are GPCRs which are related to odor-sensing, they respond to stimuli with their own ligands and send signals to olfactory neuron. There are olfactory receptors which are known to respond to biomarkers and odorants related to meat deterioration. By utilizing olfactory receptors and analyzing their reaction pattern to VOCs, difference of odor and quality can be monitored.

Three human olfactory receptors, OR2J2, OR2W1, and TAAR5, are known to react with octanol, hexanal, and trimethylamine (TMA), respectively [12][13]. These receptors are known to detect odor and taste molecules from food, and compatible for detection spoilage of food [14]. Alcohols and aldehydes, like octanol and hexanal are produced by lipid oxidation/degradation [15]. TMA is usually formed by microbial activity and proteolytic activity [16].

Two trace amine-associated receptors (TAARs) in zebrafish, TAAR13c and TAAR13d are known to bind selectively to cadaverine and putrescine, which are death-associated odorants [17]. Those compounds are related to stinky odor of deteriorated meat. These receptors were applied to bioelectronic nose for monitoring of food spoilage [18]. They were produced in form of receptor-embedded nanodiscs and their functionalities to bind to ligands were well-defined (Figure 1.1.).

Thus, five receptors above were selected to monitor quality of vacuum-packed meat. Those receptors were produced in *Escherichia coli* (*E. coli*) systems because of its economic advantages,

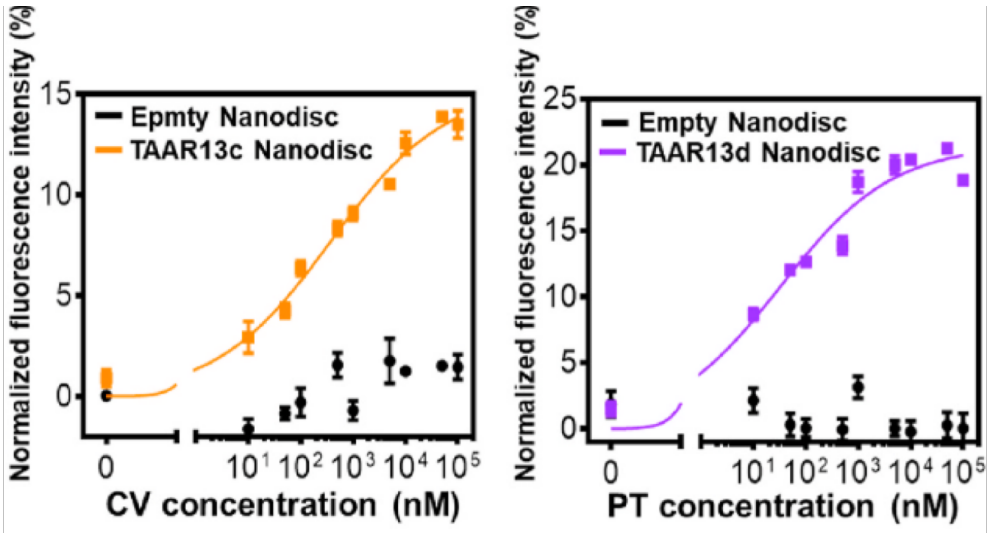

Figure 1.1. Responses of TAAR13c and TAAR13d nanodiscs to their ligands [18]– those nanodiscs showed responses to their ligands, cadaverine and putrescine.

good production yield, and well-studied expression control. When GPCRs are produced in *E. coli* system, they used to be produced as inclusion bodies, which are not folded properly. Reconstitution of structure to attain functionality is essential to utilize receptors [19]. Reconstitution techniques such as embedding in detergent micelles and usage of nanodiscs have been studied intensively [20]. Nanodiscs have the best effectiveness as reconstitution material thanks to their stability of structure [21]. Nanodisc is disc form mimicry of lipid bilayer environment. It allows transmembrane proteins to be reconstituted right alike its native structure in cell membrane. Receptor-embedded nanodisc insists of receptor, lipids, and membrane scaffold protein (MSP) (Figure 1.2.). GPCRs are known to be refolded and maintain their functionality in nanodiscs [22][23]. They assemble into nanodiscs by self-assembly induced with removal of detergent from mixture of them. Consequently, the receptor-embedded nanodiscs are used for monitoring of meat deterioration.

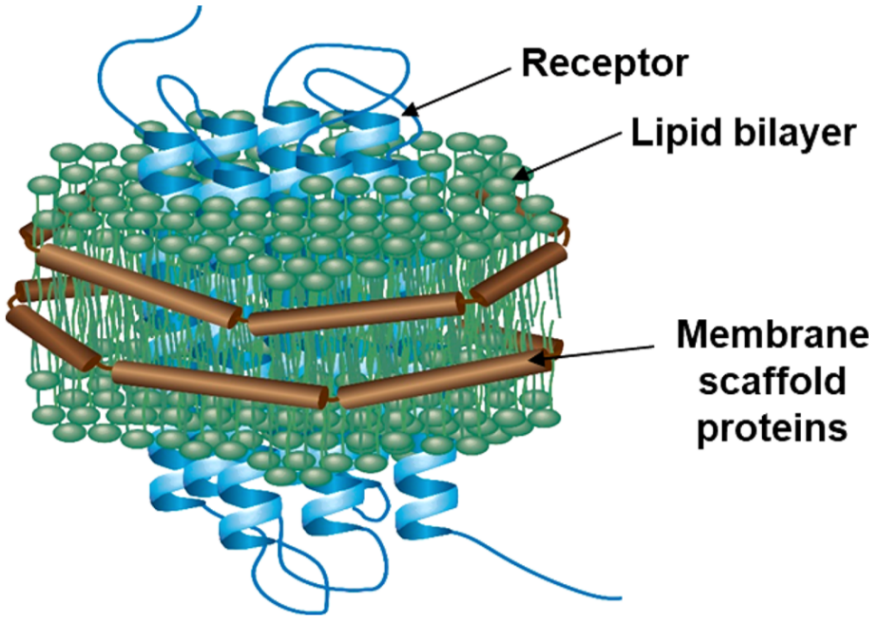

Figure 1.2. Structure of receptor embedded nanodiscs - protein is embedded in lipid bilayer which mimics cell membrane

Chapter 2. Materials and methods

2.1. Production of receptor-embedded nanodisc

2.1.1. Purification of olfactory receptors

Genes of OR2J2, OR2W1, and TAAR5 receptors were cloned in bacterial expression vectore pET-DEST42 (Invitrogen), and rraA gene, which are used for overexpression of receptors was cloned in bacterial expression vector pBAD33.1 (Receptech). Those vectors were transformed in Rosetta^{TM} 2 *E. coli* strain (Merck). After transformation, *E. coli* was incubated in 100 μg/mL ampicillin and 40 μg/mL chloramphenicol LB agar plates for 16 h at 37℃. In 5 mL LB media containing 100 μg/mL ampicillin and 40 μg/mL chloramphenicol, a single colony from transformed cell plate was inoculated. It was incubated for 16 h at 37℃. Then it was moved to 250 mL LB media and incubated. Incubated bacteria were inoculated into 1 L LB media containing 0.2% arabinose, then incubated at 30℃. When OD600 value was 0.4~0.5, 1 mM IPTG was added to the medium to induce the expression, and incubation at 25℃ were maintained for 16 h, Cells were harvested by centrifugation (4℃, 7000 g, 15 min). Cells were resuspended in PBS buffer containing 2 mM EDTA (pH 7.4). Resuspended cell was sonicated (5 seconds pulse on/off, 38% amplitude, 5 min) then centrifugated (4℃, 12000 g, 30 min). Insoluble fraction was collected and solubilized with solubilization buffer (0.1 M Tris- HCl, 20 mM SDS, 1 mM EDTA, 0.1 M DTT, pH 8.0) and incubated at 30℃, overnight. The solubilized proteins were centrifugated (20℃, 12 000 g, 30 min) and the supernatant was packed in dialysis

membrane (MEMBRA-CEL®, 14 kDa cutoff) and dialyzed with binding buffer (0.1 M sodium phosphate, 10 mM SDS, pH 8.0). The olfactory receptors were purified by HisTrap™ HP column (GE Healthcare) with washing buffer (0.1 M sodium phosphate, 10 mM SDS, pH 7.0) and elution buffer (0.1 M sodium phosphate, 10 mM SDS, pH 6.0). The purified olfactory receptors were desalted and changed its containing buffer to HEPES I buffer (20 mM HEPES, 100 mM NaCl, 25 mM cholate, pH 8.0) by HiTrap™ Desalting column (GE Healthcare).

2.1.2. Purification of membrane scaffold protein

Rosetta 2 cells were transformed with pET-28a vector containing MSP1E3D1 and cultured in LB agar plate containing 50 μg/mL kanamycin. A single colony was collected and incubated in 5 mL LB medium containing 50 μg/mL kanamycin for 16 ho, 37℃. Then it was moved to 250 mL LB medium and incubated overnight. Then it was inoculated to 1 L LB media and incubated at 37℃ until the OD600 value reached 0.4~0.5. Then it was induced by 1 mM IPTG and incubated for 4 h. Cells were centrifuged (7000 g, 20 min, 4℃) and harvested. Harvested cells were resuspended and lysed in binding buffer (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, pH 8.0). Then lysate was filtered with 0.45 μm filter. Filtered lysates were purified by HisTrap™ HP column (GE healthcare) with washing buffer (20 mM Tris-HCl, 50 mM imidazole and 0.5 M NaCl, pH 8.0) and elution buffer (20 mM Tris-HCl, 350 mM imidazole and 0.5 M NaCl, pH 8.0) Purified protein was desalted in HEPES II buffer (20 mM HEPES- NaOH, 100 mM NaCl, 25 mM cholate, pH 8.0) by HiTrap™ Desalting column. Afterwards, His-tag on MSP were truncated by TEV protease (1:50 TEV to MSP molar ratio), for 4 h, at room temperature. Then His-tag cleaved MSP were collected by reverse purification with HisTrap™ HP column.

2.1.3. SDS-PAGE analysis for receptors

20 μL of protein samples were inserted into polyacrylamide gel and analyzed by SDS-PAGE and western blot. After gel electrophoresis, gel was put in staining solution (Coomassie Blue 0.5 g/L, acetic acid 7% (v/v), methanol 40% (v/v)) for 1 h at room temperature. Then it was destained by destaining solution I (acetic acid 7% (v/v), methanol 40% (v/v)) and destaining solution II (acetic acid 7% (v/v), methanol 5% (v/v)) for 1 h, 16 h, respectively. Western blot was tried with anti-His tag mouse antibody (Santa Cruz Biotechnology) as primary antibody. HRP-conjugated goat anti-mouse antibody (Milipore) was used as the secondary antibody.

2.1.4. Assembly and purification of receptor-embedded nanodiscs

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was selected as the lipid for nanodisc assembly. Lipids were purified and dissolved in HEPES I buffer. Lipids were frozen and thawed by sonicating at 70℃, repeated for 3 times. Then MSP and lipids were mixed. After 10 min, receptors were added to mixture of MSP and lipids. The mixture was incubated in 25℃ for 2 h. After then, Bio-Beads (Bio-Rad) were added to the mixture to remove any detergents (cholate, SDS) for 16 h. The mixture was purified by size exclusion chromatography (SEC) with Superdex 200 Increase 10/300 GL column (Cytiva). SEC enables removal of larger aggregates from nanodiscs. The column was equilibrated with HEPES II buffer and nanodiscs with proper size were collected and stored at -80℃ deep freezer.

2.1.5. Western blot for nanodiscs

20 μL of protein samples were inserted into polyacrylamide gel

and analyzed by western blot. Western blot was tried with anti-V5 epitope rabbit antibody (Santa Cruz Biotechnology) as primary antibody. HRP-conjugated goat anti-rabbit antibody (Millipore) was used as the secondary antibody.

2.2. Characterization of receptor-embedded nanodiscs

2.2.1. Dynamic light scattering

Dynamic light scattering (DLS) was carried out to determine sizes of nanodiscs. Sizes of nanodiscs were determined by size distribution by number, obtained by Malvern Zetasizer Ultra (Malvern Panalytical). Its data was analyzed by ZS Xplorer software (Malvern Panalytical).

2.2.2. Tryptophan queching assay

To confirm functionalites of receptor-embedded nanodiscs; OR2J2, OR2W1, and TAAR5 nanodiscs, tryptophan quenching assay was tried. Tryptophan quenching assay is assay which tryptophan quenching assay was tried using spectrofluorometer (LS 55 Lumincscence Spechrometer, PerkinElmer). Selection of wavelength was 280 nm for excitation and 334 nm emission slit. The normalized fluorescence intensity ($\Delta F/F_0$) was calculated as $\Delta F/F_0$ (%) = $[(F_0 - F)/F_0]$ x 100 (%). Where F_0 is fluorescence intensity of reactant untreated nanodiscs and F is fluorescence intensity of reactant treated nanodiscs after consideration of matrix effect.

2.3. Preparation and physicochemical analyses of vacuum-packed meat

2.3.1. Preparation of vacuum-packed meat

3 kg of grade 1 *Longissimus Lumborum* castrated cow beef was prepared to be wet-aged 3 days after slaughtered. It was 2.5 cm thick and cut into loaves. To control difference comes from fat content, storage duration of each loaf. All loaves of beef except control sample (0 day stored sample) were vacuum-packed and stored in 2℃ refrigerator. On every 14 days after the experiment start (storage duration 14, 28, 42, 56, 70 days), vacuum-packed meat for each day was unpacked and pH and total aerobic bacteria were measured. Meat samples for volatile basic nitrogen quantification, TBARS assay, and tryptophan quenching assay with receptor-embedded nanodiscs were prepared as ground meat after measuring its pH and stored in -70℃.

2.3.2. Total aerobic bacteria enumeration

27 mL of saline (0.85% NaCl) was added to 3 g of ground meat samples. After 10-fold serial dilution, the diluted samples were spread on plate count agar (Difco Laboratories, USA). Bacteria were enumerated after 72 h incubation at 37℃.

2.3.3. pH measurement

pH of vacuum-packed meat was determined directly with handheld type pH meter after calibration of pH meter.

2.3.4. Volatile basic nitrogen quantification

27 g of Distilled water was added to 3 g sample, then homogenized for 30 sec. Homogenized samples were centrifugated (2265 xg, 4℃, 10 min) and filtrated by Whatman No. 1 filter paper. After then, 0.01 N boric acid 1 mL, and indicator (0.066% methyl red in ehtnaol: 0.066%

bromocresol green in ethanol=1:1) 100 μL was put in inner chamber of Conway. One side of outer chamber of Conway, 50% potassium carbonate 1 mL was added, and filtrated sample 1 mL was added on another side. Sealed the chamber and reacted them for 1 h at 37℃. Solution in inner chamber was titrated by 0.01 N HCl. To calculate VBN content, $\text{VBN (mg\%)} = 0.14^{*} \times (\text{added HCl solution volume (mL)} - \text{added HCl solution volume for control (mL)}) \times \text{dilution factor} \times 100$ (* VBN amount equivalent to 0.01 N HCl solution 1 mL) was used.

2.3.5. TBARS assay

In 5 g sample, 15 mL of distilled water was added. To prevent additional oxidization, 7.2 butylated hydroxyl toluene in ethanol was added. The sample was homogenized for 30 min. after then, in 15 mL tube, 0.5 mL of homogenized sample and 20 mM 2-thiobarbituric acid in 15% trichloroacetic acid (TBA/TCA) 1 mL were added and reacted in 90℃ water bath, and centrifugated (2265 xg, 4℃, 10 min). Supernatants of samples were collected and the absorbance at 532 nm wavelength was measured by plate reader. The TBARS was calculated by formula

$$\text{TBARS (mg MDA/sample kg)} = (\text{Absorbance of sample} - \text{Absorbance of blank well}) \times 5.58$$

2.4. Monitoring of quality of vacuum-packed meat with receptor-embedded nanodiscs

2.4.1. vacuum-packed meat sample preparation

Meat samples taken after 0, 14, 28, 42, 56, 70 days of storage were ground and stored at -70℃ deep freezer. Before tryptophan quenching assay, 3 g of meat samples were mixed in 27 g water (10 wt%). Mixed samples were vortexed for 30 seconds, then extraction and inversion (30 rpm) was performed for 1 h, at 4℃. After extraction,

supernatant of sample was filtrated with filter paper, and they were filtered with Amicon® filter with cutoff 10 kDa (Sigma-Aldrich) by centrifugation (10 min, 4000 rpm, 4℃). Total 6 batches of samples were used for the experiment.

2.4.2. Tryptophan quenching assay

Tryptophan quenching assay with OR2J2, OR2W1, TAAR5, TAAR13c, and TAAR5 nanodiscs was performed with meat sample prepared in section 2.4.1. Experimental conditions were same as condition written in section 2.2.3.

2.4.3. Principal Component Analysis of tryptophan quenching assay results

Tryptophan quenching assay results were statistically analyzed with principal component analysis based on R, thanks to MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml).

Chapter 3. Results and discussion

3.1. Production of receptor-embedded nanodiscs

Receptors which are newly tried to be in nanodiscs, OR2J2, OR2W1, TAAR5 were produced well in *E. coli* system. Production of receptors at proper size was confirmed in SDS-PAGE and western blot. Molecular weights of OR2J2, OR2W1, and TAAR5 are 35.2 kDa, 36.1 kDa, and 38.2 kDa, respectively. OR2J2, showed a band at 30 kDa in Coomassie blue gel staining. OR2W1 also showed clear one band at 32 kDa. TAAR5 showed dimer band at 80 kDa (Figure 3.1.). In western blot image, OR2J2 showed same band at 30 kDa, and dimer band at 60 kDa. OR2W1 showed band at 32 kDa and some bands at 70~90 kDa region, which can be interpreted as dimer or trimer bands. TAAR5 showed band at 45 kDa, and its dimer band was at 75 kDa (Figure 3.2.). Through this experiment, all the receptors were determined to be purified clearly.

Nanodiscs were assembled with not only 3 receptors produced in this study but also additional 2 receptors, TAAR13c and TAAR13d which were already produced. They were already confirmed to be in right condition before its use. After nanodisc assembly, they were purified and isolated from aggregates by SEC. After all production and purification steps were over, western blot took place to confirm proper embedding of receptors. In western blot image (Figure 3.3.), OR2J2 nanodiscs showed band at same size as its receptor has shown. OR2W1 nanodiscs showed monomer band at 35 kDa, dimer band at 65 kDa. TAAR5 showed monomer band at 38 kDa, and dimer band at 80 kDa. There are intensive bands at 25 kDa, and 50 kDa, they are bands of untruncated MSP. Besides, western blot image of TAAR13c showed

monomer band at 45 kDa, and dimer band at 75 kDa. TAAR13d also showed monomer band at 45 kDa and dimer band at 75 kDa. By western blot analysis of nanodiscs, embedding of receptors in nanodiscs is confirmed.

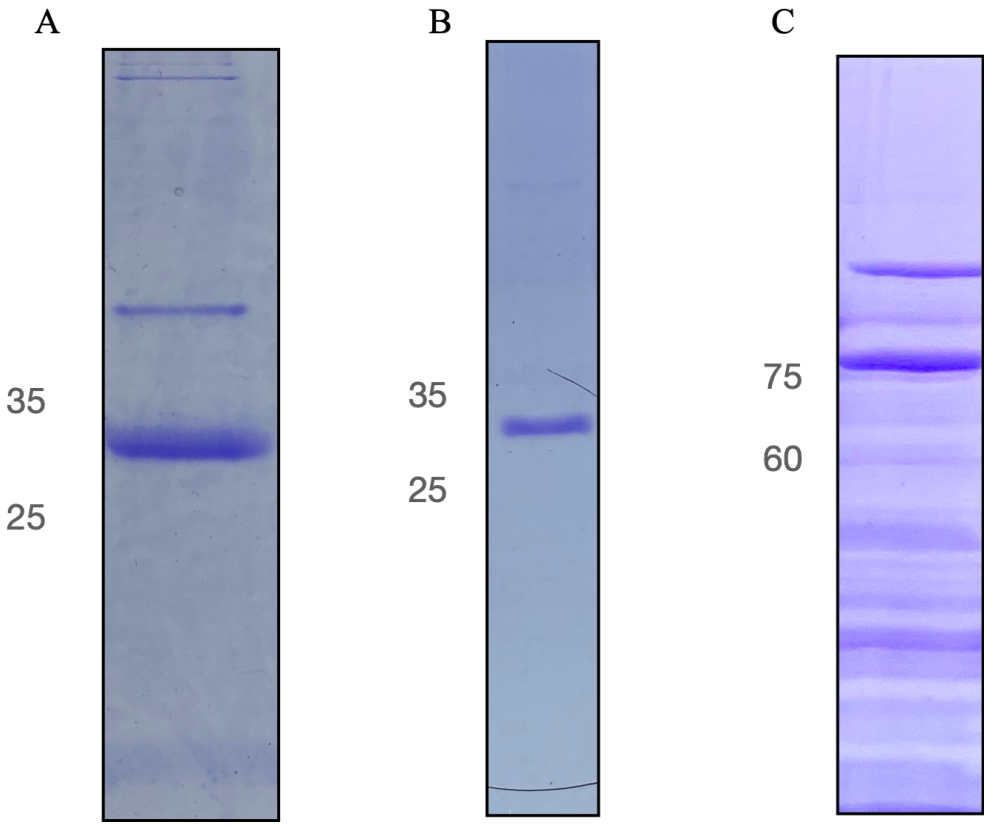

Figure 3.1. Coomassie blue staining result of receptors – Those receptors are OR2J2 (A, 35.2 kDa), OR2W1 (B, 36.1 kDa), and TAAR5 (C, 38.2 kDa). They have clear bands at 30 kDa, 32 kDa, and 75 kDa, respectively.

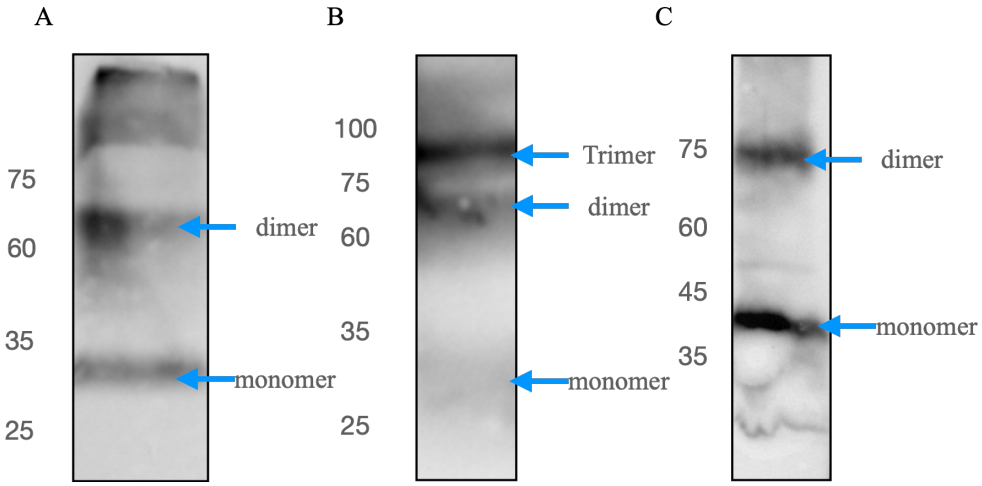

Figure 3.2. Western blot results of receptors – Those receptors are OR2J2 (A, 35.2 kDa), OR2W1 (B, 36.1 kDa), and TAAR5 (C, 38.2 kDa). They showed bands at right molecular weight, respectively.

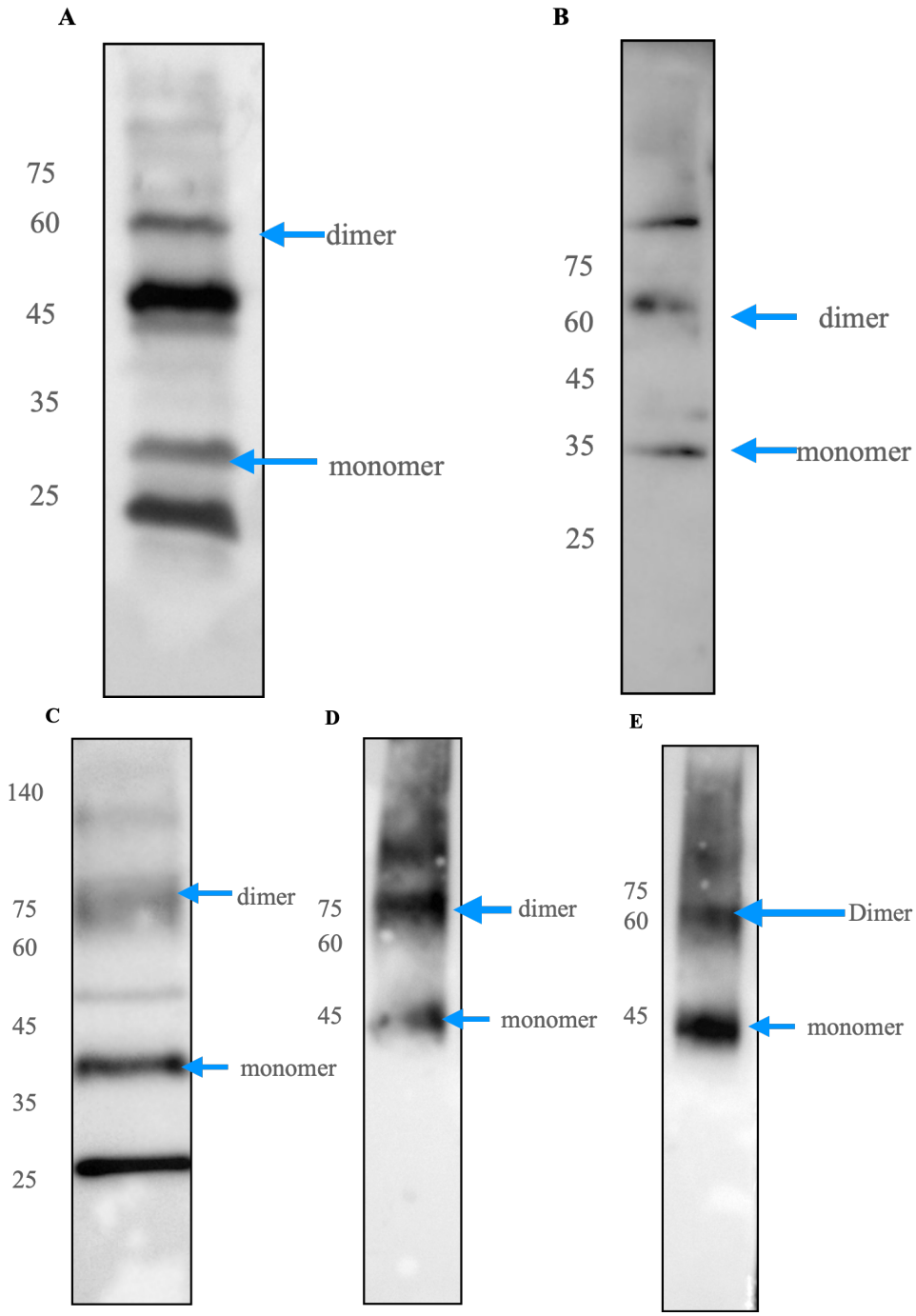

Figure 3.3. Western blot results of receptor-embedded nanodiscs- Olfactory receptors, OR2J2 (A, 35.2 kDa), OR2W1 (B, 36.1 kDa), TAAR5 (C, 38.2 kDa), TAAR13c (D, 38.4 kDa), and TAAR13d (E, 38.6 kDa) were embedded in nanodiscs and its existance in nanodiscs was confirmed by western blot.

3.2. Characterization of receptor-embedded nanodiscs

DLS is size determination method used for particles in solution. It was carried out to determine size of purified receptor-embedded nanodiscs. In nanodisc purification steps, size exclusion chromatography is essential to isolate nanodiscs from aggregates which are distinctly bigger than nanodiscs. Range of diamter of nanodiscs assembled with MSP1E3D1 is known to be about 9.8 to 17 nm [24]. DLS data (Fig 3.4.) shows that the receptor-embedded nanodiscs were produced in right size. Peak diameters determined by DLS were 9.4, 9.3, 11.4, 8.2, and 12.2 nm for OR2J2, OR2W1, TAAR5, TAAR13c, and TAAR13d nanodiscs, respectively. Some of them seems slightly smaller than range of nanodisc diameters, but there is no possible smaller product than nanodiscs. Thus, the receptor-embedded nanodiscs were determined to be isolated well from aggregate by confirming their sizes are in right range.

Tryptophan quenching assay is method to determine binding affinity of ligands and proteins. Proteins have intrinsic fluorescence due to their aromatic residues, phenylalanine, tyrosine, and tryptophan. Tryptophan has the strongest fluorescence and spectral character, so disturbance of their fluorescence can be observed easily. Herein, binding of ligand and structural change of protein makes chemical potential around tryptophan residues usually covered in transmembrane domain, it reduces fluorescence of tryptophan residues. This is called as tryptophan quenching. Binding affinity of ligands to proteins/receptors can be determined by observing intensity of tryptophan quenching [25].

Tryptophan quenching assay was carried out to confirm functionalities of unfunctionalized receptor-embedded nanodiscs;

OR2J2, OR2W1, and TAAR5 nanodiscs, repeated 3 times for each nanodiscs. Receptor embedded nanodiscs showed dose-dependent reactivity in tested range against their ligands; octanol, hexanal, and trimethylamine, respectively (Fig 3.5.).

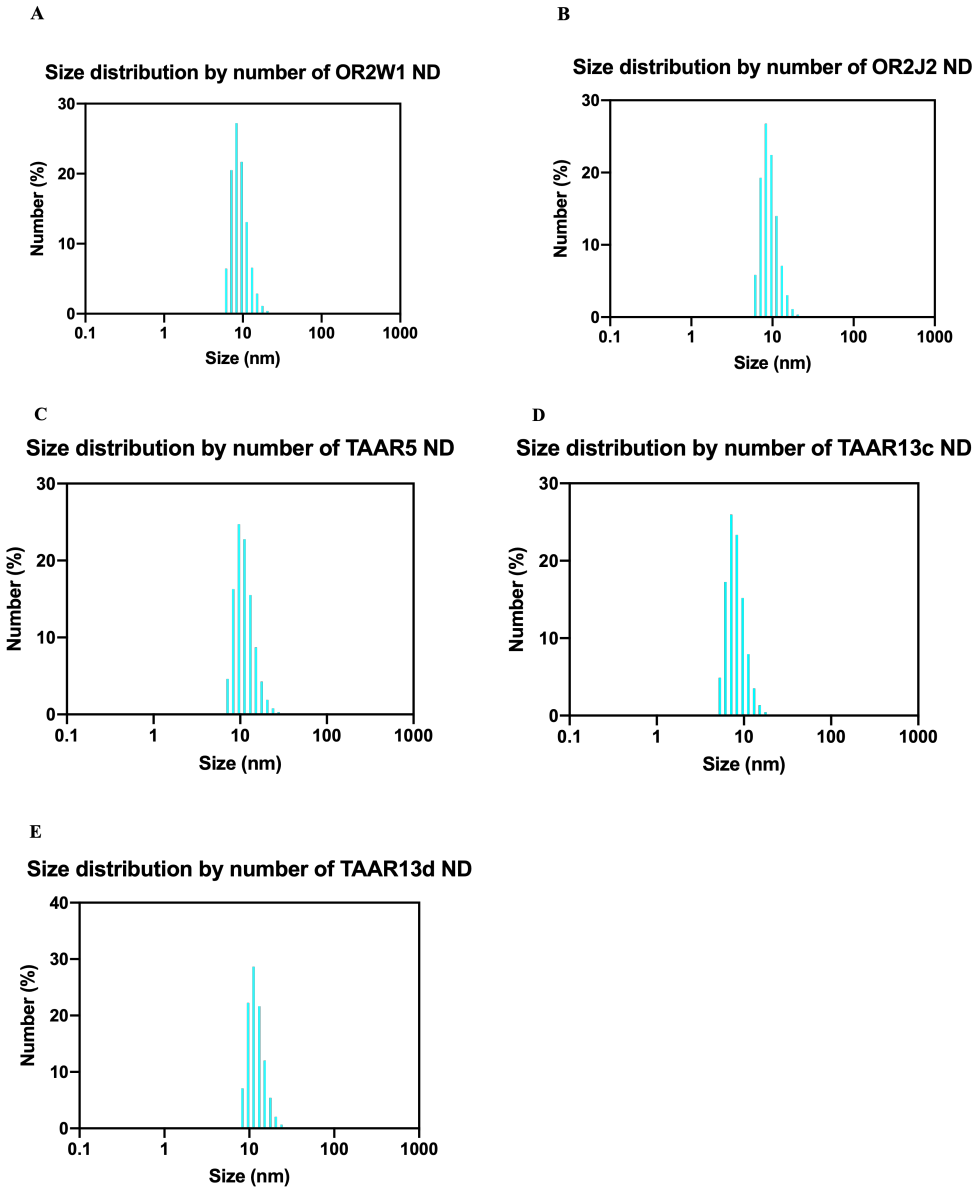

Figure 3.4. Dynamic light scattering results of receptor-embedded nanodiscs - Receptor embedded nanodiscs (OR2J2 (A), OR2W1 (B), TAAR5 (C), TAAR13c (D), and TAAR13d (E) nanodiscs) were properly isolated from aggregates.

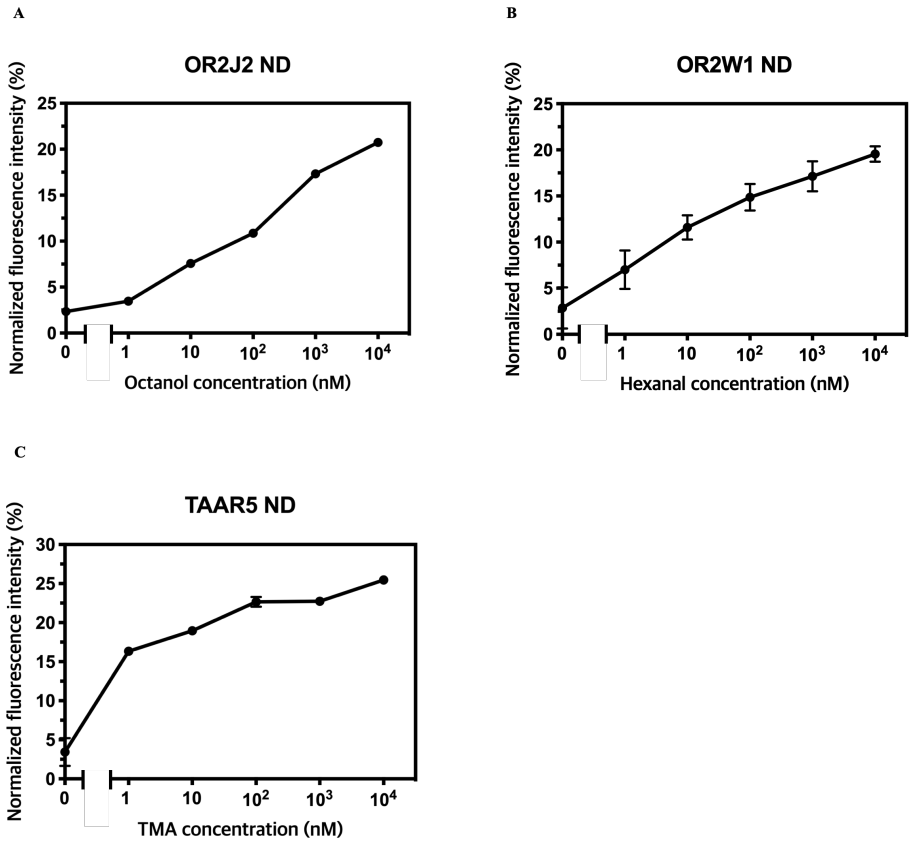

Figure 3.5. Tryptophan quenching assay results of receptor-embedded nanodiscs with their ligands - Functionalities of OR2J2 (A), OR2W1 (B), and TAAR5 (C) nanodiscs were confirmed by tryptophan quenching assay, they bound with their ligands in dose-dependent manner.

3.3. Physicochemical analysis of vacuum-packed meat

Vacuum-packed meat was prepared and stored well, and their physicochemical properties were obtained by various assays (TAB, pH, VBN content, TBARS) (Figure 3.6.Table 3.1.).

Bacterial count is one of the most major determinants of deterioration of meat. When the bacterial count of meat exceeds 7.0 log CFU/g, the meat is considered to be spoiled [26]. In this case, total aerobic bacterial count of vacuum-packed meat stored for more than 56 days exceeded 7 log CFU/g (Figure 3.6. A).

pH of the meat is known to be increase during storage in normal meat products by the formation of basic nitrogen compounds. However, pH of vacuum-packed meat is decreased during storage. This might be translated as lactic acid accumulation produced as fermentation product of anaerobic bacteria [27] (Figure 3.6. B).

VBN content is also one of the determinants of meat deterioration. When the VBN content of meat is greater than 20 mg% (red borderline), the meat is considered to be deteriorated. VBN content of vacuum-packed meat increased by time and skyrocketed when the storage duration is longer than 56 days, which are exceed or just on the borderline (Figure 3.6. C).

TBARS is carried out to quantify malonaldehyde (MDA) content, which can be indicator of lipid oxidation. MDA amount was increased until 28 days, but after then it decreased (Figure 3.6. D). MDA amount can be decreased or degraded by time goes on, or it can react with several compounds such as amino acids and urea [28][29] .

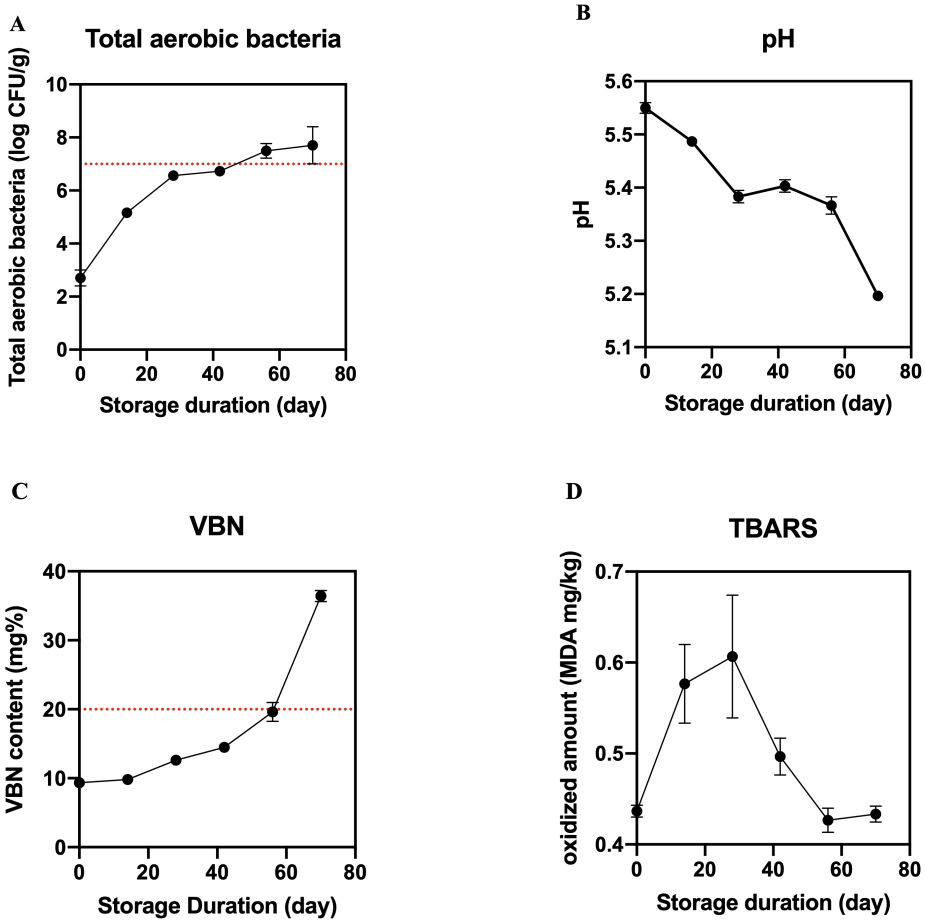

Figure 3.6. Physicochemical assay results of vacuum-packed meat by storage duration- TAB (A), pH (B), VBN (C), and TBARS (D) were measured for vacuum-packed meat samples.

Traits[1]	Storage duration (days)						SEM[2]
	0	14	28	42	56	70	
TAB (log CFU/g)	2.70[d]	5.16[c]	6.56[b]	6.73[b]	7.49[ab]	7.71[a]	0.204
pH	5.55[a]	5.49[b]	5.38[c]	5.40[c]	5.37[c]	5.20[d]	0.011
TBARS (MDA mg/kg)	0.44[b]	0.58[ab]	0.61[a]	0.50[ab]	0.43[b]	0.43[b]	0.035
VBN (mg%)	9.33[d]	9.80[d]	12.60[cd]	14.47[c]	19.60[b]	36.40[a]	0.713

Table 3.1. Quality traits of vacuum-packed meat stored for 70 days- Quality traits by storage duration are described in table

[a-d]Different letters within the same row indicate a significant difference ($p < 0.05$).

[1]TAB, total aerobic bacteria; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; VBN, volatile basic nitrogen.

[2]Standard error of means (n=18).

3.4. Monitoring of quality of vacuum-packed meat with receptor-embedded nanodiscs

Vacuum-packed meat samples were prepared as described. Tryptophan quenching assay of receptor-embedded nanodiscs was carried out with vacuum-packed meat samples. (Figure 3.7.)

Empty nanodiscs were used as control group. They didn’t show any notable reaction with vacuum-packed meat (Figure 3.7. A).

However, reactivitites of receptor-embedded nanodiscs differed by storage duration, and their trend of reaction differed by receptors. Receptor-embedded nanodiscs used for tryptophan quenching assay are known to be respond to odorants related to meat deterioration. Increment of their reactivity can be interpreted as development of odor related to meat deterioration (Figure 3.7, B~F).

Reaction pattern of receptor-embedded nanodiscs were anlyzed and visualized by PCA (Figure 3.8.). As the time goes on, centroid of each group moves to right area on PC plane, and the direction overlapped well with vector of TAAR13c and OR2W1 (Figure 3.8. B), those receptors can be considered as crucial receptors of reaction pattern change. Especially, samples stored for 56, 70 days, which were determined as deteriorated, had no overlap with 95% confidence area of samples stored for 0, 14 days on PC plane. That is, reaction pattern change displayed on PC plane can indicate deterioration of meat.

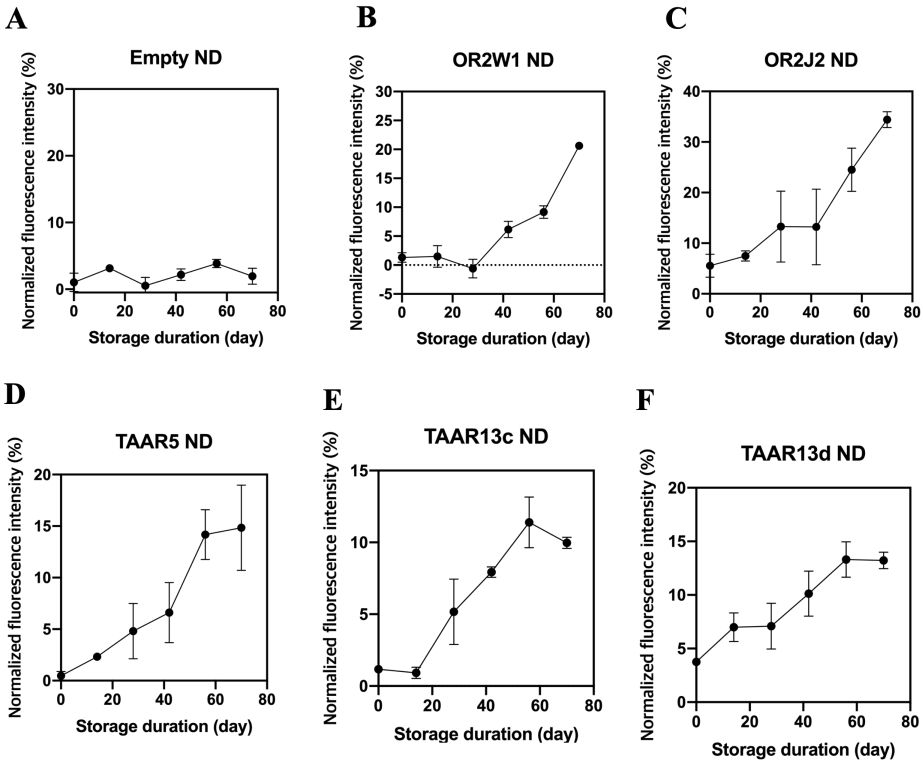

Figure 3.7. Tryptophan quenching assay results of receptor-embedded nanodiscs with vacuum-packed meat - Empty nanodisc (A) didn’t showed notable reaction. Receptor-embedded nanodiscs (B~F, OR2J2, OR2W1, TAAR5, TAAR13c, and TAAR13d, respectively) showed reactivity change as time goes by.

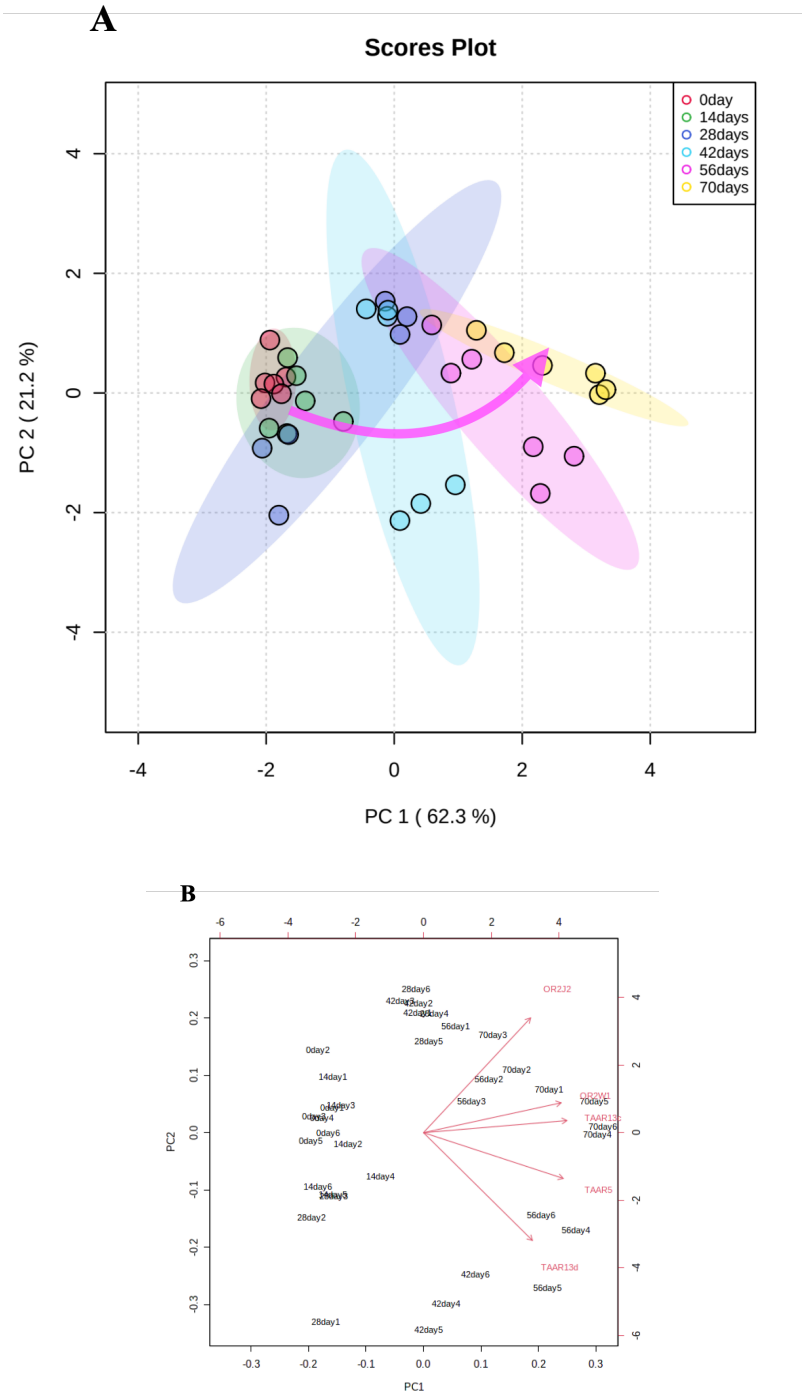

Figure 3.8. Pattern change visualization by principal component analysis- Reaction pattern attained by PCA differed by storage duration, and centroids seemed to be move to right (A). Projection of unit vector of receptors reaction are described as red arrows (B).

Chapter 4. Conclusion

Olfactory receptor-embedded nanodiscs were appied for monitoring of vacuum-packed meat deterioration. Olfactory receptors were selected by its known ligands. OR2J2, OR2W1, and TAAR5 were known as receptors bind to deterioration-related odorants. They were produced well in *E. coli* system and their purification was confirmed by Coomassie blue staining and western blot.

On the other hands, TAAR13c and TAAR13d were already confirmed their functionalities as embedded in nanodiscs. Three receptors written above, and these two receptors were embedded to nanodiscs and its embedding and structural character was confirmed by western blot and dynamic light scattering. OR2J2, OR2W1, TAAR5 nanodiscs were confirmed to have functionality by tryptophan quenching assay with their known ligands.

Vacuum-packed meat samples were prepared varying storage duration (0, 14, 28, 42, 56, 70 days), physicochemical analyses of them were carried out to determine deteriorated point, and it was 56 days. Tryptophan quenching assay of receptor embedded nanodiscs with vacuum-packed meat samples was carried out and reaction pattern change was observed by the storage duration differ. Pattern change was visualized and analyzed by PCA and compared with physicochemical analyses results. As a result, reaction pattern change of olfactory receptor embedded nanodiscs is confirmed to be related to deterioration. Thus, olfactory receptor-embedded nanodiscs are potential material for quality monitoring of vacuum-packed meat.

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초록

고기는 부패하기 쉬운 식품으로써, 그 부패에 대한 모니터링이 필수적이다. 그래서 부패의 감지나 모니터링을 위한 방식으로 여러 이화학적 검사들과 화학 물질들에 대한 분석이 진행된다. 한편, 진공포장육은 그 저장 기간 중 발생하는 숙성을 통한 향미 증진과 더불어 경제적인 측면 등에서의 다양한 이점으로 인해 육류 회사에서 자주 쓰이는 식육의 저장 방식이다. 반면에, 이러한 진공포장육의 부패나 품질에 대한 규격이나 표준이 아직 존재하지 않는다. 진공포장육의 물성과 미생물학적 성질이 함기포장된 육류와 다르기 때문이다. 그래서 진공포장육의 품질은 경험과 관능에 의존하여 평가하게 된다. 이를 모방하고 대체하기 위하여 후각 수용체를 삽입한 나노디스크를 이용하여 시료에 대한 반응성을 확인하고 패턴화함으로써 시료의 상태를 확인할 수 있도록 하였다. OR2J2, OR2W1, 그리고 TAAR5 를 *E. coli* 에서 생산한 뒤 나노디스크에 삽입되었음을 확인하였다. 이후 후각 수용체 삽입 나노디스크들의 냄새 물질에 대한 반응성을 트립토판 소광 실험을 통하여 확인할 수 있었다. 마지막으로 기능성이 확인된 나노디스크들에 진공 포장시킨 뒤 저장한 고기 시료를 처리하여 그들의 반응 패턴이 변화함을 트립토판 소광 실험을 통하여 확인하였으며, 주성분 분석을 통하여 반응 패턴의 변화를 시각화하였고, 그 결과 이화학적 분석 결과를 통해 밝혀낸 부패한 시료들(56, 70일 간 저장)에서의 반응 패턴 변화가 가장 컸음을 알 수 있었다. 결론적으로, 후각 수용체 삽입 나노디스크를 이용하여 진공포장육의 품질 변화를 모니터링할 수 있었다.

Keywords : Olfactory receptor, Nanodisc, Vacuum-packed meat, Meat deterioration, Food quality monitoring

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