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Research

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Evaluation of influenza-specific immunoglobulin Y stability in liquid, solid, and cream-based body care formulations

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Abstract

Background Specific immunoglobulin Y (IgY) is widely used in immunotherapy, with expanding applications in body care products. Topically applied influenza-specific antibodies may provide an alternative strategy for preventing respiratory infections.

Objective This study aimed to evaluate the stability and biological activity of influenza-specific IgY in various body care products.

Methods The biological activity of influenza-specific IgY was evaluated in various body care products at different concentrations. Commercial face mists contained 5% and 10% w/v IgY, face mists and nasal sprays contained 0.5% w/v IgY, lip balm, sunscreen, and hand cream contained 0.1% and 0.25% w/v IgY. The products were stored at room temperature for four weeks, and organoleptic changes were monitored weekly. IgY activity was assessed by enzyme-linked-immunosorbent-assay (ELISA).

Results In both face mist products, the aroma gradually became fishy, the color remained stable, and turbidity increased. The biological activity of IgY was still detectable by ELISA at concentrations of 0.5%, 5%, and 10%. In nasal spray, no changes in aroma, color, or turbidity were observed, but IgY activity was not detectable. In lip balm, sunscreen, and hand cream, only slight color changes occurred without any change in aroma, and IgY activity was not detected.

Conclusion Influenza-specific IgY retained biological activity in face mist formulations at higher concentrations (≥0.5%) despite observable changes in aroma and turbidity. In contrast, IgY activity was not detected in nasal sprays, lip balm, sunscreen, or hand cream, suggesting that the formulation type and IgY concentration significantly influence its stability and detectability in body-care products.

Keywords biological activity | body care products | ELISA | influenza-specific Immunoglobulin Y | organoleptic stability

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Introduction

Acute respiratory tract infection (ARI) is a condition that affects the alveoli and is characterized by symptoms such as a runny nose, cough, and fever, typically lasting up to two weeks (Cindy *et al.*, 2024). ARI is classified into upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI) (Oktaria *et al.*, 2021). URTIs can be caused by either bacteria or viruses; however, the majority of ARIs are viral in origin (Correia *et al.*, 2021). According to a 2018 report by the National Health Research and Development Agency (Riskesdas), the prevalence of ARI in Indonesia reached 9.3%, with the highest rates found in East Nusa Tenggara (15.5%), Papua (13.1%), West Papua (12.3%), Banten (11.9%), and Bengkulu (11.8%) (Kemenkes, 2018). Among the viral causes of ARI, influenza virus is of particular global concern because of its impact on both human and animal health. Influenza viruses are associated with high morbidity and mortality rates and contribute to a significant socioeconomic burden (Asha & Kumar, 2019). Previous influenza pandemics have been attributed to strains such as H1N1, H2N2, and H3N2 (Soema *et al.*, 2015).

Viral infections can generally be prevented by vaccination or treatment with antiviral agents or antibodies. Specific antibodies have been widely used in research, diagnostics, and immunotherapy (Yakhkeshi *et al.*, 2025). However, their potential applications continue to expand, with one emerging trend being their incorporation into skincare products (Tsukamoto *et al.*, 2018) One type of antibody which often produced for immunotherapeutic use is immunoglobulin Y (IgY), derived from poultry. IgY, which is naturally present in egg yolk, offers a strategic potential for passive immunity-based immunotherapy. Its applications include use as an oral dietary supplement (El-Kafrawy *et al.*, 2023), oral treatment for dental caries and oral infections (Poetri *et al.*, 2006; Poetri *et al.*, 2008; Bachtiar *et al.*, 2019), and topical formulations for skin care and treatment of skin infections (Tsukamoto *et al.*, 2018)

IgY formulated as a spray or topical product for application to body surfaces, such as the face and hands, shows promise as an alternative strategy for preventing viral infections, particularly those that target the respiratory tract. Body care products such as face mists, nasal sprays, lip balms, hand creams, and sunscreens containing influenza-specific IgY could form part of a broader infection mitigation approach. These formulations are expected to inhibit viral entry through the respiratory tract, thereby contributing to control of viral transmission. However, the virus-neutralizing effect of a specific IgY depends on the preservation of its biological activity after formulation. Therefore, this study aimed to evaluate the stability of the biological activity of IgY following its incorporation into body-care products.

Methods

Ethical clearance

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Biomedical Sciences, IPB University (Approval No. 014/KEH/SKE/XI/2020; November 3, 2020).

Influenza-specific immunoglobulin Y

The IgY used in this study was prepared as a freeze-dried powder and isolated from egg yolks of laying hens immunized with the FLUBIO® influenza vaccine (Biofarma, Bandung, Indonesia). This influenza-specific immunoglobulin Y was prepared from a previous study by Saputri *et al.* (2024), who produced IgY from layers vaccinated with a quadrivalent influenza vaccine, and by Rachmawan *et al.* (2025), who compared four purification methods to identify the most effective and feasible technique for large-scale IgY production.

A preliminary experiment was conducted to prepare IgY solutions at concentrations ranging from 0.1% w/v to 10% w/v, followed by evaluation using an enzyme-linked immunosorbent assay (ELISA) with ID Screen® Influenza A Antibody Competition Multi-species kit (IDvet, Grabels, France) to determine the lowest detectable concentration. The IgY concentration for the final product formulation was selected on the basis of these preliminary findings. Preliminary results showed that IgY at a concentration of 0.1% could still be detected by ELISA. Therefore, in this study, the IgY concentrations used were 10%, 5%, 0.5%, 0.25%, and 0.1%.

Body care product preparation

In this study, five body care formulations–face mist, nasal spray, lip balm, sunscreen stick, and hand cream–were aseptically prepared. All products were stored in sterile containers at room temperature, shielded from direct light, and observed for four weeks.

We used two types of face mist in this study: a commercial Pixy Aqua Beauty Protecting Mist®, Indonesia spiked with IgY at 5% and 10% w/v in 20 mL aliquots, and a laboratory prepared face mist composed of rose hydrosol (60 mL), distilled water (57 mL), 0.6 g leucidal, and 2.4 g vegetable glycerin (Rahmadasmi *et al.*, 2024), into which IgY was added at 0.5% w/v (0.5 g), resulting in a final volume of 100 mL. The nasal spray formulation consisted of 0.162 g NaCl and 3.6 mL NaOCl topped to 10 mL with distilled water, to which IgY was added at 0.5% w/v.

A lip balm blend was prepared by weighing 8 g of carnauba wax, 16 g of shea butter, and 16 mL of avocado oil into a sterile, heat-resistant vessel, following the method described by Hanum *et al.* (2021), with modifications using only three ingredients and the addition of shea butter. The mixture was gently melted

by microwaves until it was fully liquefied. The molten oil phase was immediately transferred to a water bath and stirred continuously during cooling. When the temperature reached 60°C, before any visible sediment appeared, freeze dried IgY powder was added aseptically at concentrations of 0.10% and 0.25% w/v (1.0 mg and 2.5 mg IgY per 10 mL, respectively). Stirring was continued until the mixture appeared homogeneous and free of sediments, poured into sterile lip balm tubes, and allowed to solidify undisturbed for 5 min.

For the sunscreen stick, an anhydrous wax–butter matrix was assembled by melting 4 g beeswax, 6.5 g shea butter, 3 g cocoa butter, and 8 mL coconut oil, followed by uniform dispersion of 5 g zinc oxide at elevated temperature (Dube & Masresha, 2022). Once the molten blend cooled to approximately 56°C, freeze dried IgY was incorporated at concentrations of 0.10% and 0.25% w/v, ensuring minimal thermal stress to preserve antibody activity. The homogeneous mixture was poured into tubes and allowed to solidify naturally over 6–12 hours.

The hand cream was formulated by separately heating the aqueous phase (35 g aqua bidest, 20 g rose water, 2 g aloe vera gel, 2 g glycerin) and oil phase (10 g grapeseed oil, 10 g sunflower oil, 2 g BTMS 50, 4 g cetyl alcohol, approximately 0.5 g vitamin E oil) in a 75°C water bath with intermittent microwave cycles (Sousa *et al*., 2018). After 5–minute of medium-speed mixing as the blend cooled, the temperature was checked periodically and IgY (0.10% and 0.25% w/v) was gently introduced at ≈ 40°C, then stirred to yield a stable, non-separated emulsion.

Organoleptic observations

Sensory characteristics were assessed weekly over four weeks. For liquid products, such as face mist and nasal spray, changes in color, odor, and turbidity were recorded; for solid and cream formulations, such as lip balm, sunscreen stick, and hand cream, we evaluated color, odor, and consistency. Any deviations in color, odor, turbidity (for liquids) (Wan *et al.*, 2025), or consistency were noted descriptively at each time point. Color variation was assessed with reference to the "Shades of White" chart (HTML Color Codes: https://htmlcolorcodes.com/colors/shades-of-white/) and (Cohen *et al*., 2023).

Biological activity evaluation

For the biological activity test, samples from each formulation were collected weekly and stored appropriately until the analysis. For face mist and nasal spray, 0.8 mL aliquots were aseptically collected each week and stored in microtubes at -20°C. For lip balm and sunscreen stick, a 0.5 cm section was cut weekly using a knife sanitized with 70% ethanol between cuts to prevent cross-contamination. The sections were then placed in 2 mL microtubes, labeled, and stored at -20°C. Similarly, for the hand cream, 1 mL samples were collected weekly and stored in microtubes at -20°C until biological activity testing was conducted.

The biological activity of IgY in these products was assessed using the enzyme-linked immunosorbent assay (ELISA) method with the ID Screen® Influenza A Antibody Competition Multi-species kit (IDvet, Grabels, France) following the manufacturer's instructions. This competitive ELISA detects antibodies against the nucleoprotein of the Influenza A virus across multiple species. A positive ELISA result indicates that the IgY in the product retains biological activity as it reacts with the influenza virus antigen coated in the ELISA kit.

Results

Organoleptic stability

Color changes of body care products. The color changes in the four IgY-based liquid products over a four-week storage period revealed distinct stability patterns (**Table 1**). Both the commercial face mists FM1 and FM2 consistently maintained a cream hue from week 0 to week 4, indicating excellent chromatic stability. In contrast, laboratory-prepared face mist FM3 initially appeared colorless at week 0, transitioned to ivory by weeks 1 and 2, and then reverted to cream by week 3 through week 4. The nasal spray NS likewise started with cream coloration, shifted to beige during weeks 2 and 3, and then returned to cream by week 4. These observations suggest that while the commercial formulations are visually stable, the lowerconcentration, lab-prepared products show transient color evolution, which is potentially reversible as a function of storage time.

Table 1 Color changes of liquid product of body care formulations

Weeks	FM1	FM2	FM3	NS
0	cream	cream	colorless	cream
1	cream	cream	ivory	cream
2	cream	cream	ivory	beige
3	cream	cream	cream	beige
4	cream	cream	cream	cream

FM1: commercial face mist with 5% w/v IgY; FM2: commercial face mist with 10% w/v IgY; FM3: face mist with 0.5% w/v IgY; NS: nasal spray with 0.5% w/v IgY.

Over the 4-week storage period, the visual appearance of various IgY-based solid and cream products displayed distinct patterns of color evolution (**Table 2**). Both lip balm formulations LP1 and LP2 remained off-white up to week 2, but gradually shifted toward an off-white beige hue by weeks 3 and 4. Both sunscreen formulations, SS1 and SS2, consistently retained an off-white coloration throughout the entire period, exhibiting high visual stability. Both hand cream formulations, HC1 and HC2, maintained a uniform milky white shade across all weeks, indicating strong chromatic robustness. These observations suggest that among the tested formulations, lip balms exhibited modest color shifts over time, whereas creams and sunscreens demonstrated notable stability.

Table 2 Color changes of solid and cream product of body care formulations

Weeks	LP1	LP2	SS1	SS2	HC1	HC2
0	off-white	off-white	off-white	off-white	milky white	milky white
1	off-white	off-white	off-white	off-white	milky white	milky white
2	off-white	off-white	off-white	off-white	milky white	milky white
3	off-white beige	off-white beige	off-white	off-white	milky white	milky white
4	off-white beige	off-white beige	off-white	off-white	milky white	milky white

LP1: lipbalm with 0.1% w/v IgY; LP2: lipbalm with 0.25% w/v IgY; SS1: sunscreen with 0.1% w/v IgY; SS2: sunscreen with 0.25% w/v IgY; HC1: handcream with 0.1% w/v IgY; HC2: handcream with 0.25% w/v IgY.

Turbidity changes of liquid body care products. Over the four-week stability study, the four IgY-based liquid products demonstrated distinct and time-dependent changes in opacity (**Table 3**). Commercial face mist FM1 consistently retained a *slightly turbid* appearance from week 0 to week 4, indicating minimal but stable light scattering. In contrast, FM2 was *turbid* throughout the study period, reflecting an inherently higher baseline opacity. The laboratory-prepared face mist FM3 began as *clear* on day 0, progressed to *slightly turbid* in weeks 1 and 2, and became fully *turbid* by weeks 3 and 4, indicating a gradual increase in light-scattering particulates or colloidal formations. Similarly, the NS nasal spray transitioned from *slightly turbid* at week 0 to *turbid* at week 1, maintaining that state through the end of week 4.

Consistency changes of solid and cream body care products. Throughout the 4-week observation period, no notable changes in consistency were detected in either solid (lip balm and sunscreen) or cream (hand cream) formulations containing IgY at concentrations of 0.1% or 0.25% w/v. All solid products (LP1, LP2, SS1, and SS2) consistently retained a slightly dense texture from week 0 to week 4, while both cream formulations (HC1 and HC2) maintained a soft consistency over the same period. The changes in consistency are presented in **Table 4**. These findings indicate that the presence of IgY, regardless of its concentration, did not affect the physical consistency of the products during storage at room temperature.

Odour changes of body care products. During the 4-week storage period, liquid products containing IgY showed varying degrees of odor change, depending on the formulation type and IgY concentration. Both the commercial face mists FM1 and FM2 developed a faint fishy odor as early as week 1, which persisted without further intensity changes through week 4. Similarly, the non-commercial face mist FM3 also exhibited a faint fishy odor from week 1; however, by week 4, the odor intensified to a fishy smell. In contrast, nasal spray NS remained odor-free ("clean") throughout the observation period. The odor changes are shown in **Table 5**. These results suggest that the emergence of a fishy odor in IgY-containing liquid products may be influenced by formulation type and matrix composition rather than solely by IgY concentration.

Over the 4-week storage period, no changes in odor were detected in either lip balm formulation (LP1 and LP2) or hand cream (HC1 and HC2), all of which consistently retained a neutral scent. In contrast, both sunscreen formulations, SS1 and SS2, exhibited a fruity scent from the start of the observation period, which

Table 3 Opacity changes of liquid product of body care formulations

Weeks	FM1	FM2	FM3	NS
0	slightly turbid	turbid	clear	slightly turbid
1	slightly turbid	turbid	slightly turbid	turbid
2	slightly turbid	turbid	slightly turbid	turbid
3	slightly turbid	turbid	turbid	turbid
4	slightly turbid	turbid	turbid	turbid

FM1: commercial face mist with 5% w/v IgY; FM2: commercial face mist with 10% w/v IgY; FM3: face mist with 0.5% w/v IgY; NS: nasal spray with 0.5% w/v IgY.

Table 4 Consistency changes of solid and cream product of body care formulations

Weeks	LP1	LP2	SS1	SS2	HC1	HC2
0	a bit dense	a bit dense	a bit dense	a bit dense	soft	soft
1	a bit dense	a bit dense	a bit dense	a bit dense	soft	soft
2	a bit dense	a bit dense	a bit dense	a bit dense	soft	soft
3	a bit dense	a bit dense	a bit dense	a bit dense	soft	soft
4	a bit dense	a bit dense	a bit dense	a bit dense	soft	soft

LP1: lipbalm with 0.1% w/v IgY; LP2: lipbalm with 0.25% w/v IgY; SS1: sunscreen with 0.1% w/v IgY; SS2: sunscreen with 0.25% w/v IgY; HC1: handcream with 0.1% w/v IgY; HC2: handcream with 0.25% w/v IgY.

Table 5 Odour changes of liquid product of body care formulations

Weeks	FM1	FM2	FM3	NS
0	clean	clean	clean	clean
1	fainty fishy	fainty fishy	fainty fishy	clean
2	fainty fishy	fainty fishy	fainty fishy	clean
3	fainty fishy	fainty fishy	fainty fishy	clean
4	fainty fishy	fainty fishy	fishy	clean

FM1: commercial face mist with 5% w/v IgY; FM2: commercial face mist with 10% w/v IgY; FM3: face mist with 0.5% w/v IgY; NS: nasal spray with 0.5% w/v IgY.

remained unchanged through week 4. The fruity scent of sunscreen originates from mango butter, which is one of its key components. The odor changes are shown in **Table 6**. These findings indicate that IgY incorporation at the tested concentrations did not induce off-odors in solid or cream products, and that the characteristic fruity scent of the sunscreen formulations was stable over time.

Biological activity of influenza specific IgY in body care products

The presence of influenza-specific IgY was assessed in various personal care formulations over a 4-week storage period (**Table 7**). Influenza-specific IgY was consistently detected in all commercial and laboratory-prepared FM1, FM2, and FM3 face mists at every time point (weeks 0–4), indicating the presence of a stable antibody in these liquid formulations. In contrast, IgY was not detected at any time point in the NS nasal spray or in any of the solid and cream formulations, including lip balm (LP1, LP2), sunscreen (SS1, SS2), and hand cream (HC1, HC2). These results suggest that formulation type plays a critical role in maintaining the detectability of influenza-specific IgY, with liquid face mist formulations showing superior IgY stability compared to nasal spray, solid, and cream products at room temperature.

Discussion

This study assessed the incorporation of influenza-specific IgY into body care products by evaluating organoleptic stability parameters, such as color, turbidity, consistency, and odor, and determined whether the antibody retained its biological activity after formulation. These results indicated that both the stability and biological detectability of influenza-specific IgY were strongly influenced by the formulation matrix. Among the tested products, commercial face mists (FM1 and FM2) exhibited superior stability, maintaining consistent color and turbidity throughout storage, likely because of optimized excipient systems containing stabilizers, antioxidants, and surfactants. In contrast, laboratory-prepared face mist (FM3) and nasal spray (NS) showed temporary increases in turbidity and slight color shifts, suggesting partial protein aggregation or excipient–protein interactions in less-stabilized matrices. The nasal spray initially appeared cream, shifted to beige during weeks 2–3, and reverted to cream by week 4, possibly due to reversible physical processes, such as sedimentation or re-dispersion of dispersed particles, or minor excipient-related chemical interactions (Ehrick *et al.*, 2013). Solid and cream formulations demonstrated greater physical

Table 6 Odour changes of solid and cream product of body care formulations

Weeks	LP1	LP2	SS1	SS2	HC1	HC2
0	neutral scent	neutral scent	fruity scent	fruity scent	neutral scent	neutral scent
1	neutral scent	neutral scent	fruity scent	fruity scent	neutral scent	neutral scent
2	neutral scent	neutral scent	fruity scent	fruity scent	neutral scent	neutral scent
3	neutral scent	neutral scent	fruity scent	fruity scent	neutral scent	neutral scent
4	neutral scent	neutral scent	fruity scent	fruity scent	neutral scent	neutral scent

LP1: lipbalm with 0.1% w/v IgY; LP2: lipbalm with 0.25% w/v IgY; SS1: sunscreen with 0.1% w/v IgY; SS2: sunscreen with 0.25% w/v IgY; HC1: handcream with 0.1% w/v IgY; HC2: handcream with 0.25% w/v IgY.

Table 7 Detection of IgY spesific influenza in the product of body care formulations

Weeks	FM1	FM2	FM3	NS	LP1	LP2	SS1	SS2	HC1	HC2
0	+	+	+		-	-	-	-	-	-
1	+	+	+	-	-	-	-	-	-	-
2	+	+	+	-	-	-	-	-	-	-
3	+	+	+	-	-	-	-	-	-	-
4	+	+	+	-	-	-	-	-	-	-

(-): IgY spesific influenza was not detected in product; (+): IgY spesific influenza was detected in product; FM1: commercial face mist with 5% w/v IgY; FM2: commercial face mist with 10% w/v IgY; FM3: face mist with 0.5% w/v IgY; NS: nasal spray with 0.5% w/v IgY; LP1: lipbalm with 0.1% w/v IgY; LP2: lipbalm with 0.25% w/v IgY; SS1: sunscreen with 0.1% w/v IgY; SS2: sunscreen with 0.25% w/v IgY; HC1: handcream with 0.1% w/v IgY; HC2: handcream with 0.25% w/v IgY.

stability, with only minor changes observed in lip balms, indicating that low IgY concentrations (≤0.25%) did not affect the structural integrity of lipid- or emulsion-based systems. Variations in the stability of liquid products may result from differences in excipients and solvents, which influence protein–excipient interactions (Ohtake *et al.*, 2011). Proteins are generally more stable in solid and cream formulations, in which excipients can directly stabilize proteins through molecular binding in the dry state (Ohtake *et al.*, 2011).

Changes in odor were observed primarily in the liquid formulations, with FM1, FM2, and FM3 developing a fishy smell during storage, whereas the nasal spray and solid/cream products remained unchanged. This phenomenon may be attributed to protein hydrolysis or oxidative degradation in aqueous environments, whereas lipid-rich matrices suppress odor release. Although these findings suggest that liquid formulations offer superior protein stability, further optimization is required to improve sensory acceptability, for example, through odor-masking agents, antioxidants, or IgY encapsulation strategies. Odor changes may occur due to protein denaturation, which can be triggered by destabilizing excipients such as sugars (Jacob *et al.*, 2006), for example, glycerin is present in FM3. Therefore, it is crucial that proteins are encapsulated to minimize denaturation in the formulation (Cicerone *et al.*, 2015).

The instability of IgY observed in the nasal spray formulation may be attributed to the presence of sodium hypochlorite (NaOCl), a strong oxidizing agent capable of damaging protein structures. NaOCl is widely recognized for its ability to inactivate enveloped viruses. However, its antimicrobial activity primarily depends on the concentration of undissociated hypochlorous acid (HOCl), as the hypochlorite ion (OCl^-) is considerably less effective (Giarratana *et al.*, 2021). HOCl acts by oxidizing sulfhydryl groups and interfering with glucose metabolism, amino acid decarboxylation, and protein synthesis while also reacting with nucleic acids to cause microbial death (EPA, 2011). It effectively disrupts cell membranes, cytoplasmic components, and DNA integrity (Block & Rowan 2020). At an acidic pH, HOCl penetration into microbial membranes increases, enhancing its biocidal effect (Hricova *et al.*, 2008). In a recent study, HOCl-containing solutions were shown to inactivate more than 99.8% of SARS-CoV-2 within one minute (Giarratana *et al.*, 2021). These findings support the hypothesis that oxidative reactions mediated by NaOCl or HOCl may lead to IgY denaturation, fragmentation, or epitope destruction, thereby reducing the antibody detectability in nasal spray formulations.

Conversely, excipients such as glycerin and lipids might exert protective effects on protein stability depending on their concentration and formulation environment. The presence of glycerin in face mist formulations likely contributed to the preservation of IgY stability by reducing aggregation and conformational stress during storage. Glycerol stabilizes proteins by promoting compact native conformations through electrostatic and amphiphilic interactions at hydrophobic surfaces, thereby preventing unfolding and aggregation (Vagenende *et al*., 2009). This mechanism supports the observed maintenance of color and turbidity in glycerin-containing formulations, suggesting that glycerin acts as a protective co-solvent that enhances IgY structural integrity in aqueous environments. Lipid-based matrices in creams or balms may enhance protein stability by creating a hydrophobic barrier that limits aqueous-mediated degradation of sensitive biomolecules. In cosmetic systems, lipids function as carriers that reduce water contact and improve the physicochemical stability of the active ingredients (Ahmad & Ahsan, 2020).

In this study, all products were stored at room temperature based on the assumption that body care products are generally kept under ambient conditions during typical consumer use. The detection of influenza-specific IgY in the liquid formulations throughout the four-week observation period indicated that IgY remained stable at room temperature. This finding aligns with previous reports demonstrating that IgY exhibits relatively high thermal stability compared to mammalian IgG, and can retain its antigen-binding activity for several weeks at ambient temperatures when properly formulated with stabilizing excipients (Carlander *et al.*, 2000; Schade *et al.*, 2005).

Biological activity, as assessed by influenza-specific IgY detection, was preserved only in the face mist formulations (FM1, FM2, and FM3) but not in the nasal spray or solid/cream products. The lack of detectability in nasal spray may be related to incompatible excipients, ionic strength, or pH conditions that destabilize the antibody. Hypochlorite in the nasal spray may induce IgY denaturation or fragmentation by cleaving peptide bonds, thereby reducing antigen-binding capacity. Such peptide bond disruption has been reported during HOCl interactions in aqueous media and in reactions between egg proteins and sodium hypochlorite (Andrés *et al.*, 2022). Similarly, in solid and cream formulations, IgY may have been denatured during processing or entrapped within the lipid matrix, thereby reducing its accessibility for detection. Alternatively, because of either low IgY concentration or poor re-dissolution in the aqueous phase, ELISA detection is limited. ELISA was selected as the primary method for assessing the biological activity of influenza-specific IgY based on its high sensitivity, specificity, and suitability for detecting antigen–antibody interactions in complex formulations. However, it does not directly measure the functional neutralizing activity. Therefore, while consistent ELISA positivity indicates structural preservation of antigen-binding epitopes, it may not fully represent the biological potency of the antibody in vivo. Complementary assays, such as virus neutralization or hemagglutination inhibition tests, are required to confirm the functional activity (Carlander *et al.*, 2000; Mine & Kovacs-Nolan, 2002).

Although numerous studies have examined antibody stability in pharmaceutical formulations, research on the incorporation of antibodies, particularly avian IgY, into cosmetic-type products remains scarce. Previous studies have primarily focused on IgY preservation in aqueous buffers or food matrices under controlled storage conditions (Schade *et al.*, 2005; Mine & Kovacs-Nolan, 2002). Other studies have reviewed multiple factors influencing mAb instability, such as formulation, environmental, and handling conditions, and emphasized the need for comprehensive, multi-technique physicochemical analyses to accurately assess mAb stability and the validity of extended stability claims (Le Baslé *et al.*, 2020). In contrast, the present study investigated IgY stability in multi-component cosmetic vehicles, including emulsions, lipid-based matrices, and surfactant-containing liquids, under ambient conditions typical of consumer storage.

This study had several limitations that should be acknowledged. First, the observation period was limited to four weeks under a single ambient storage condition, which may not accurately represent the long-term or stress stability profiles. Second, the organoleptic evaluations were qualitative and did not include quantitative analytical methods, such as spectrophotometry or rheological measurements. Third, the assessment of biological activity relied solely on ELISA, which detects antibody presence but does not confirm functional efficacy. Variations in excipient composition between commercial and laboratory-prepared formulations may also have contributed to differences in stability outcomes. Furthermore, the current study focused only on the physicochemical stability of IgY within formulations and did not extend to *in vivo* or animal model testing. Consequently, the safety and potential applicability of these IgY-based products for human use remains to be established.

Overall, these findings highlight that the choice of formulation matrix plays a critical role in preserving the stability and activity of IgY in cosmetic products. Face mist formulations have emerged as the most promising delivery platform, maintaining IgY detectability while requiring further refinement to address sensory limitations, such as odor and turbidity. In contrast, solid and cream products demonstrated excellent physical stability but poor antibody detectability, indicating the need for novel stabilization techniques, such as encapsulation, lyophilization, and the use of protective excipients. Thus, this study extends antibody stability research beyond biomedical formulations, providing a new foundation for the development of antibody-based cosmetic and personal care products. Future studies should extend the storage period and include stress conditions, such as temperature, light, and humidity variations, to comprehensively assess formulation stability. Incorporating standardized formulation protocols and functional bioassays will help to verify IgY activity beyond ELISA detection and clarify the mechanisms underlying its degradation or stabilization in cosmetic-type matrices. Moreover, future research should be expanded to include toxicity evaluation, as well as *in vitro* and *in vivo* studies to confirm the safety and biological relevance of these findings for product development and consumer applications.

Conclusion

This study demonstrated that the stability and biological detectability of influenza-specific IgY in body care products is highly dependent on the formulation matrix. Face mist formulations were the most favorable platform, maintaining antibody activity while showing some sensory challenges, such as odor and turbidity, likely due to protein degradation in aqueous environments. In contrast, solid and cream products exhibited excellent physical stability but limited IgY detectability, possibly because of low concentrations or entrapment within lipid matrices. Nasal spray formulations were the least compatible, with hypochlorite-induced denaturation contributing to loss of activity. Collectively, these findings emphasize the importance of excipient selection and formulation strategies to preserve protein function. Future studies should focus on improving IgY stability and sensory properties through approaches such as encapsulation, lyophilization, or protective excipients, along with extended stability and functional bioactivity testing.

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