

Production of Functional Ice Cream Fortified by Immunoglobulin Y against *Escherichia coli* O157:H7 and *Helicobacter Pylori*

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Abstract: The production of effective functional foods that can increase public health is paramount to scientists. This study aimed to optimize methods for developing bio-ice cream comprising IgY versus *Escherichia coli* O157 and *Helicobacter pylori*. Leghorn hens were immunized intramuscularly with antigens. The egg yolk was separated from the egg, and the IgY antibody was then purified using the PEG 6000 method. Maximum IgY was reached in 2 weeks with IgY concentrations of 14.56, 11.46, 16.34, and 6.87 mg/ml for *H. pylori*, *E. coli* O157, *E. coli* + *H. pylori*, and control, respectively. The results showed that 0.6 mg/ml concentration of IgY had a significant inhibitory effect on bacteria growth using disk diffusion methods. Also, ELISA results showed that IgY activity was reliable and remained active in ice cream for 3 months. According to sensory evaluation, the global acceptance scores and the other attributes revealed the acceptable acceptance of IgY egg yolk ice cream. Consequently, it can be stated that the ice cream matrix can be considered an ideal candidate for carrying antibodies into the host body, as the specific ingredients of the ice cream matrix can appropriately protect IgY antibodies.

Keywords: IgY productions; antibody; egg yolk; functional ice cream; healthy diet; functional food.

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1. Introduction

In recent years, biologically active components have been regarded as one of the most crucial factors in food quality [1-3]. Scientific research has suggested that functional foods could be effectively used for disease prevention in humans [4-6]. However, the application of antibiotics in food-generating animals as simple candidates for this purpose has given serious cause for concern considering their potential risks to human health [7,8]. Recently, IgY or egg yolk-related antibodies have attracted scientific consideration as potential alternatives for antibiotics to encourage growth in the attendance of disease-triggering agents [9,10]. Compared to mammalian IgG, IgY has numerous benefits, including cost-effectiveness, high yield, and convenience [11]. It has been previously demonstrated that the oral consumption of IgY antibodies could provide considerable protection against various intestinal pathogens (e.g., *Helicobacter pylori*, enterotoxigenic *Escherichia coli*, *Mycobacterium tuberculosis*,

Clostridium difficile, *Salmonella typhimurium*, *Acinetobacter baumannii*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Propionibacterium acnes*, and *Vibrio* spp)[12].

E. coli O157:H7 and *H. pylori* are among the top harmful microorganisms that threaten human health [13,14]. In addition to being the well-known reason for gastritis and gastric ulcers, *H. pylori* possess a significant role in the expansion of gastric carcinomas, and about 50% of the global human population has been affected by this bacterium [15,16]. Moreover, about 10% of children with an *E. coli* O157:H7 infection will suffer from hemolytic uremic syndrome (HUS), with an annual 5% mortality rate. The children who survive will possess a higher risk for emerging chronic kidney disorder [17]. Also, it has been stated that the application of IgY versus *E. coli* O157:H7 and *H. pylori* can be predominantly valuable for diseases prevention and treatment strategies [18-20]. The growth inhibitory function of the IgY antibody is one of its vital characteristics, meaning that the IgY antibody could be intake in a daily diet with no significant side effects. Also, it could contribute to regulating the intestinal microbial population of the host [21-23]. Several studies have previously focused on adding IgY to yogurt; however, their results have not been commercialized, partly since, in their study, the type of food matrix (yogurt) reduces the concentration of antibodies [24]. Moreover, NaCl and other components of yogurt had negative effects on the stability of IgY proteins.

Hence, in the present study, the feasibility of the production of egg-ice cream containing IgY antibodies against *E. coli* O157:H7 and *H. pylori* was examined for the first time. If the results are positive and effective, this formulation can be used to develop functional ice cream and improve the health status of people in the community.

2. Material and methods

2.1. Chickens.

Sixteen white leghorn chickens 13 weeks old were purchased from a local hatcher (Animal science department of the Ferdowsi University of Mashhad). The chickens were kept under environmental conditions of 18 h light and a free diet [25].

2.2. Preparation of antigen.

E. coli O157:H7 and *H. pylori* were obtained from the Department of Microbiology of Ghaem Hospital (Mashhad, Iran). Sorbitol-MacConkey and Mueller-Hinton broth media were used for *E. coli* O157:H7 and *H. pylori* suspension. Then, 10^9 CFU/ml of each bacterium were washed with 5ml NaCl 85% (3 times) and centrifuged for 10 min at 10,000 rpm. For bacteria inactivation, pellets were suspended in 0.3% formaldehyde and then centrifuged for 10 min at 10,000 rpm. The pellets were re-suspended in 5ml NaCl 85% and stored at 4°C.

2.3. Immunization of hens.

Four groups of chickens (n=3 in each group) were immunized, and as the first immunization, they received an injection of *E. coli* O157:H7, *H. pylori*, and a combination of *E. coli* O157:H7, *H. pylori* with an equal volume of Freund's complete adjuvant (Sigma, USA) when they reached 16 weeks of age. Also, Freund's complete adjuvant without antigen was injected into the control group, followed by two booster immunizations utilizing incomplete Freund's adjuvant at a two-week interval. The last injection of chickens was done, and about a

week later, the eggs were collected daily for eight weeks, and after labeling, they were kept at 4 °C [26].

2.4. Extraction and purification of IgY.

Extraction and purification of IgY antibody from egg yolk were done using polyethylene glycol 6000 (PEG 6000) powder (Merck, Germany) according to Polson's method [26]. After separating the egg yolk, phosphate-buffered saline (twice the volume of yolk suspension, pH=7.6) was added and before mixed, followed by the addition of the PEG 3.5% (w/v). The resulting solution was shaken at room temperature for 10 minutes and finally centrifuged (13000 g, 20 min, 4 °C). To remove lipids, the supernatant was collected and filtered using Whatman filter paper. PEG 6000 was added to 8% (w/v) concentration. The shaking was then performed at room temperature for 10 minutes and centrifuged again as previously described. To dissolve the precipitation, 10 ml PBS and 12% (w/v) PEG 6000 were added and centrifuged as earlier. The pellet with IgY was re-suspended in 2 ml PBS and purified by dialysis against PBS overnight (4 °C). The IgY was investigated by the SDS-PAGE and Bradford methods.

2.5. ELISA assay for activity detection.

The specific activity of the IgY antibody against *E. coli* and *H. pylori* was examined by ELISA. 100 µL of the antigens was adsorbed on microtiter plates (10^3 , 10^4 , 10^5 , and 10^6 CFU/ml) for 1 h. The plates were then washed three times using PBS with 0.05% (v/v) Tween 20 (PBST). Using blocking buffer (1.5% BSA in PBST), nonspecific sites were blocked for 1 h at 37 °C. After being washed, we added the primary antibody, which consisted of 100 µL of diluted IgY antibody (1:2000) in PBST. Subsequently, it was incubated for 1 h at 37 °C. Afterward, the plates were re-washed, and 100 µL of rabbit anti-chicken IgY H&L (HRP) secondary antibody (Sigma, USA) at a diluted IgY antibody (1:10000) was added to each well and before incubated at 37 °C for 1 h. To perform colorimetric detection, O-phenylenediamine (OPD, Sigma) was used as a chromogenic substrate of HRP. Finally, wells were washed using PBST. In order to stop the reaction, 50 µL/well 2N H₂SO₄ was added. The absorbance of each well was measured at 492 nm [25].

2.6. Estimation of IgY cytotoxicity by disk diffusion method.

One of the simple tests for cytotoxicity of antibodies has been the investigation of bacteria growth in the presence of antibodies. The 5 different concentrations of IgY were prepared and injected into disks. 10 µl of *E. coli* and *H. pylori* suspension were cultured on specific media, and disks were placed on it. Gentamycin (10µg/ml) antibiotic was used as control.

2.7. Preparation of egg ice cream.

The ice cream preparation was done according to Herald *et al.*[27] Briefly, egg yolks containing IgY were used in the ice cream formula. 1.46 g egg yolks, 0.20 g stabilizer, 1.36 g nonfat dry milk, 6.05 g sugar, 23.58 g pasteurized milk, and 9.07 g cream were polled and stirred for 10 min at 70 °C. The suspension was cooled to 32°C and homogenized at 1500 psi. The homogenized suspension was cooled to 4°C immediately and tempered for 24 h at 4°C. Finally, ice cream was produced by freezing ice cream at -18 °C.

2.8. Investigation of the shelf life of IgY in ice cream.

The residual activity of IgY after freezing was evaluated by ELISA for 3 months. Ice cream containing IgY (5 mL) was combined with a similar amount of carbonate buffer (pH=10) and mixed for 3 min with chloroform (10 mL). The solution was centrifuged at 3000 rpm for 30 min, after which we examined the upper supernatant for IgY shelf life in ice cream using ELISA, as discussed earlier [24].

2.9. Sensory evaluation of the ice creams.

Sensory assessment of the egg yolk ice creams was conducted through surveys to evaluate consumers' level of acceptance. Within two weeks of storage, the surveys were carried out on the samples, through which participants were questioned to grade the ice creams based on color, texture, flavor, feel, taste, and global acceptance, using a 5-point hedonic scale [28].

2.10. Statistical analyses.

The results were expressed as the means \pm standard deviation (SD) for data in each shown treated group. Student t-tests were applied to recognize statistically significant differences between the two groups. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Production and purification of IgY.

The purification of IgY using the SDS PAGE methods was investigated and displayed in Figure 1.

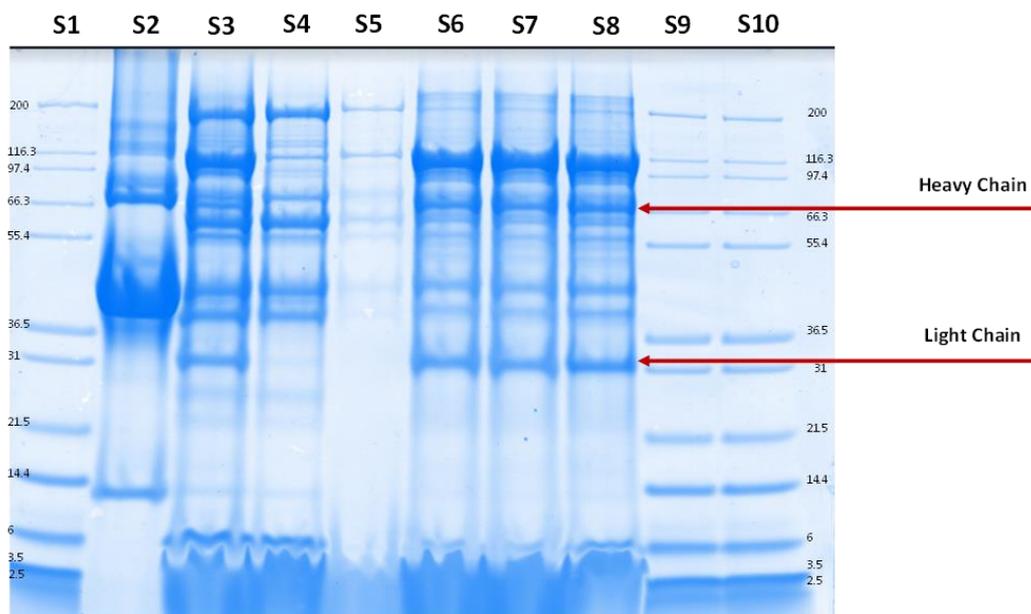


Figure 1. SDS-PAGE to assess the purity of IgY in samples. S1: Protein marker, S2: Egg white proteins, S3: Yolk proteins, S4: Yolk after 3.5% PEG 6000 extraction, S5: The IgY purification for the control sample, S6: The IgY purification for *E. coli* O157:H7 antigen sample, S7: The IgY purification for *E. coli* O157:H7 antigen sample, S8: The IgY purification for *H. pylori* antigen sample, S9 and S10: Protein marker.

Results showed that IgY purification from 4 samples (*E. coli*, *H. pylori*, *E. coli* + *H. pylori*, and control) was done correctly. The size of heavy and light chains of IgY was estimated at 67 and 35 kDa, respectively. Also, our results confirmed that IgY purity was satisfactory.

Furthermore, IgY yield in samples was 14.56, 11.46, 16.34, and 6.87 mg/ml for *E. coli*, *H. pylori*, *E. coli + H. pylori*, and control, respectively. Therefore, considering the optimal purity and high efficiency of IgY, it can be utilized as a novel biological strategy and an effective and ideal type of antibody in supplying the food safety chain in the food industry. Among the most important factors in functional food production are the yield and purification of IgY, which requires optimization for each experiment [21]. The purification of IgY from egg yolk by PEG methods is remarkably cost-effective and consists of two stages [26]. In stage 1, lipids were removed, and in stage 2, IgY was precipitated in PBS buffer. Our results showed that stage 1 had a major role in the final concentration of IgY. Amro *et al.* [21] extracted IgY using a low-pressure chromatography device and reported 3.66 mg/ml IgY for Single Comb White Leghorns egg yolk, whereas our extraction of IgY was about 2.59 mg/ml, which is suitable.

3.2. IgY activity.

ELISA assay showed binding of IgY to *H. pylori* and *E. coli* antigen that was coated on plates (Figure 2a). Besides, the outcomes discovered significant differences between control and treatment groups (P -value < 0.001). The concentration of IgY in the *E. coli + H. pylori* group was greater than that of the treatment group. Complete immunization was obtained two weeks after antigen injection (Figure 2b).

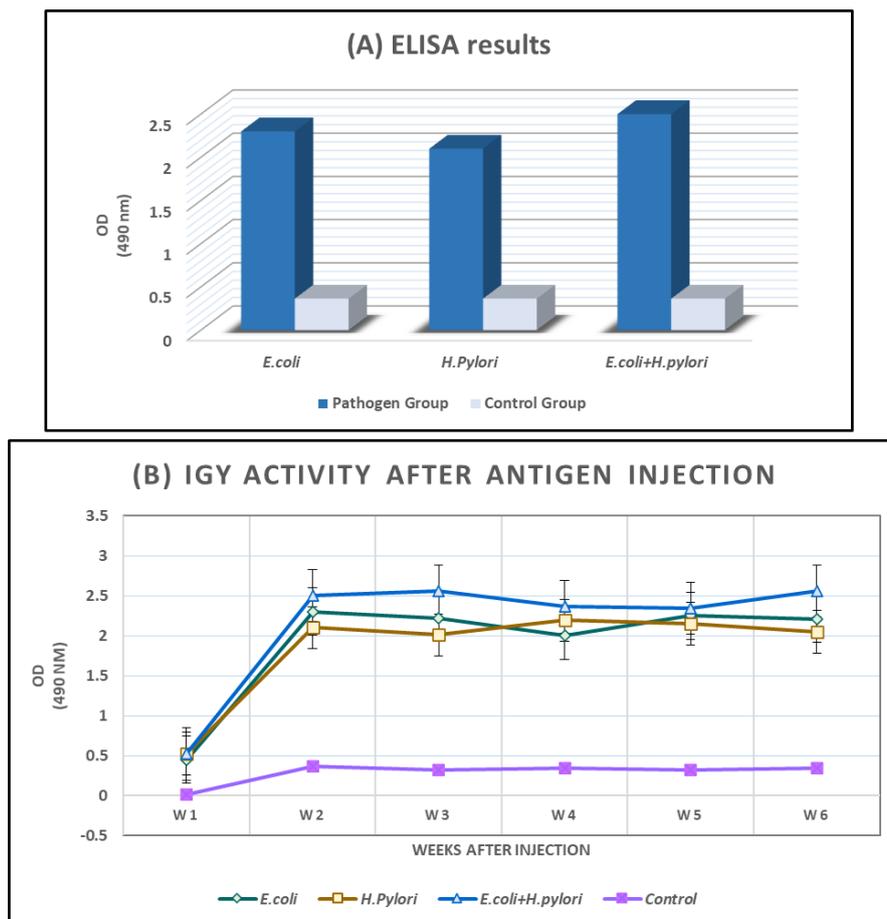


Figure 2. Results of ELISA assay for detection of IgY activity. (A) results of IgY activity; (B) Investigation of IgY activity in weeks after antigen injection.

Our results showed that the highest level of IgY purification is achieved 2 weeks after the injection. However, the decrease in IgY production was insignificant over the following

weeks. Studies showed that using an appropriate adjunct and accurate estimation of antigen concentration is crucial to the production of desired results [25,29].

3.3. Cytotoxicity of IgY.

The effect of the IgY antibody on the growth of *E. coli* and *H. pylori* was measured, and the results confirmed that, similar to gentamicin, IgY could be effective at 6 mg/ml concentration. The formation of a clear zone on the disk indicated the inhibitory effect of IgY on bacteria growth (Figure 3).

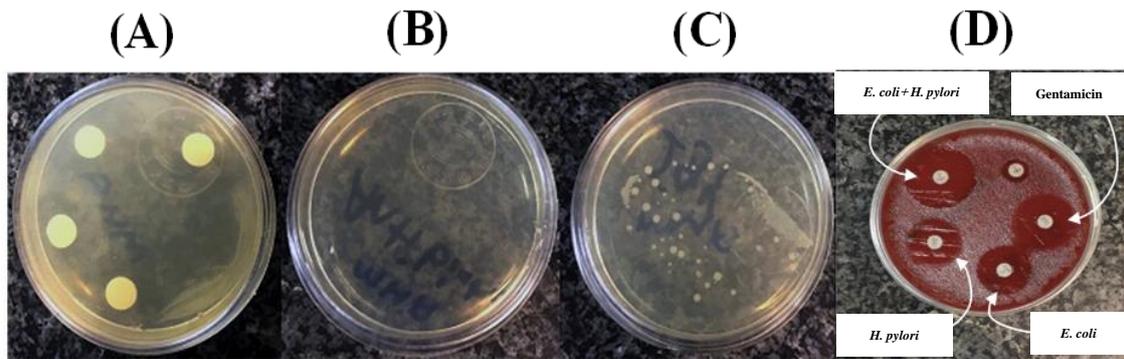


Figure 3. Investigation of IgY cytotoxicity effect on bacteria using disk diffusion methods. (A) The clear zone diameter is indicative of IgY activity. (B) *H. pylori* culture on media spread by IgY. (C) Positive control. (D) The clear zone diameter reveals anti-*E. coli* O157:H7 activity of IgY.

In light of the limited number of studies on the use of egg yolk in ice cream, our results are rather new and suggest that 0.6 mg/ml of IgY is needed for desirable effectiveness against investigated bacteria. On the other hand, pepsin and other molecules in the human stomach had an inhibiting effect on IgY activity [12]. In a study by Pauly *et al.* [26], 80% purity in the extraction of total IgY from egg yolk was achieved using PEG precipitation. Therefore, we condensed IgY by freeze-drying the egg yolks and then used it in ice cream preparation. The concentration of IgY was ~ 1.5 mg in 100 g of ice cream. ELISA analysis showed that the freeze-drying process had no significant effect on IgY activity. IgY shelf life appeared to be acceptable after 3 months, and IgY activity was not affected by time and mechanical activity.

3.4. Properties of ice cream.

The shelf life results of IgY in the matrix of ice cream exhibited that this dairy-based matrix could be most certainly applied as a novel functional food.

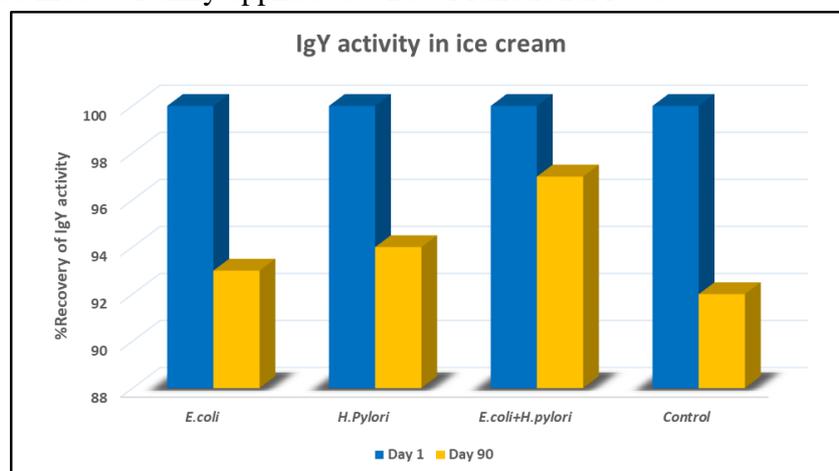


Figure 4. IgY activity in 3 months following ice cream preparation.

ELISA analysis established that IgY could remain active in the ice cream matrix for 3 months (Figure 4). The activity of IgY diminished by about 6%, which is insignificant. Nevertheless, studies have demonstrated that even broken IgY can act effectively against bacteria [10]. As we used a 1.5 mg/ml concentration of IgY in ice cream, the taste and other properties of the ice cream were not influenced.

3.5. Sensory analysis of ice cream.

A hedonic scale's sensory characteristics of ice cream demonstrated that this commodity could be an appropriate option for marketing, as no substantial alterations were detected between egg yolk and vanilla ice creams (Table 1).

Table 1. Average grades for color, flavor, taste, feel, tissue, and global acceptance for the different produced egg yolk and vanilla ice creams.

Formula	Color	Flavor	Taste	Feel	Tissue	Global acceptance
Egg yolk	3.31 ± 0.278	3.25 ± 0.251	3.43 ± 0.286	3.75 ± 0.282	3.56 ± 0.203	3.62 ± 0.21
Vanilla	3.62 ± 0.278	3.68 ± 0.251	3.312 ± 0.286	2.68 ± 0.282	3.50 ± 0.203	3.68 ± 0.21

Comparing the global acceptance scores and the other attributes revealed the acceptable acceptance of IgY egg yolk ice cream. IgY containing ice cream is deemed the foremost candidate for the formulation and production of novel functional foods. It possesses the characteristics required to preserve IgY and raise the effectiveness of IgY activity. An ideal temperature for the maintenance of ice cream was -18 °C which is utterly appropriate for IgY protection. Besides, ice cream is consumed while cold, and various studies displayed that IgY can continue effective at temperatures of up to 80 °C, and the pasteurization process did not influence IgY function [24]. However, we used pasteurized milk to tackle this problem in this study. Jaradat *et al.*[30], reported that, in the presence of carbohydrates (sucrose, lactose, and trehalose), IgY had insignificant activity. Hence, we utilized freeze-dried egg yolk to preserve IgY biological function. Consequently, it can be stated that the ice cream matrix can be considered an ideal candidate for carrying antibodies into the host body, as the specific ingredients of the ice cream matrix can appropriately protect IgY antibodies.

4. Conclusions

Our results demonstrated that pathogenic microbial antigens could stimulate a humoral immune response in Leghorn hens, resulting in a synthesis of high specific immunoglobulins against *E. coli* O157:H7 and *H. pylori*, which these specific immunoglobulins according to the ELISA assay possess a significant activity in the investigated egg ice cream matrix during 3 months. IgY can be used as a significant substitute to mammalian antibodies. It is better to immunize hens before they initiate egg-producing since the stress prompted via handling them could have a side effect on egg production, as might the nature of the antigen or adjuvant have applied. Furthermore, according to the outcomes of sensorial analysis and IgY activity in the egg ice cream, it can be concluded that the ice cream can be an appropriate candidate for transferring antibodies to the human body. It is expected that the utility of IgY technology and its widespread utilization in research and medicine will increase on a large scale. IgY is expected to play an increasingly important role in future research, diagnosis, and immunotherapy.

Units of measurement

C = Centigrade

CFU = Colony form unit

mg = Milligram

Min = Minutes

ml = Milliliter

n = Number

Psi = Pound-force per square inch

v = Volume

w = Weight

µg = Microgram

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. Abbasi, A.; Rad, A.H.; Ghasempour, Z.; Sabahi, S.; Kafil, H.S.; Hasannezhad, P.; Rahbar Saadat, Y.; Shahbazi, N. The biological activities of postbiotics in gastrointestinal disorders. *Critical Reviews in Food Science and Nutrition* **2021**, 1-22, <https://doi.org/10.1080/10408398.2021.1895061>.
2. Xu, Y.; Hamid, N.; Shepherd, D.; Kantono, K.; Spence, C. Changes in flavour, emotion, and electrophysiological measurements when consuming chocolate ice cream in different eating environments. *Food Quality and Preference* **2019**, *77*, 191-205.
3. Li, F.; Zhao, J.; Wei, Y.; Jiao, X.; Li, Q. Holistic review of polysaccharides isolated from pumpkin: Preparation methods, structures and bioactivities. *International Journal of Biological Macromolecules* **2021**, *193*, 541-552, <https://doi.org/10.1016/j.ijbiomac.2021.10.037>.
4. Lacerda, J.F.; Lagos, A.C.; Carolino, E.; Silva-Herdade, A.S.; Silva, M.; Sousa Guerreiro, C. Functional food components, intestinal permeability and inflammatory markers in patients with inflammatory bowel disease. *Nutrients* **2021**, *13*, 642, <https://doi.org/10.3390/nu13020642>.
5. Bora, P.; Ragaee, S.; Marcone, M. Characterisation of several types of millets as functional food ingredients. *International journal of food sciences and nutrition* **2019**, *70*, 714-724, <https://doi.org/10.1080/09637486.2019.1570086>.
6. Kewuyemi, Y.O.; Kesa, H.; Adebo, O.A. Trends in functional food development with three-dimensional (3D) food printing technology: prospects for value-added traditionally processed food products. *Critical Reviews in Food Science and Nutrition* **2021**, 1-38, <https://doi.org/10.1080/10408398.2021.1920569>.
7. Tang, K.L.; Caffrey, N.P.; Nóbrega, D.B.; Cork, S.C.; Ronksley, P.E.; Barkema, H.W.; Polachek, A.J.; Ganshorn, H.; Sharma, N.; Kellner, J.D. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *The Lancet Planetary Health* **2017**, *1*, 316-327, [https://doi.org/10.1016/S2542-5196\(17\)30141-9](https://doi.org/10.1016/S2542-5196(17)30141-9).
8. Doyennette, M.; Aguayo-Mendoza, M.G.; Williamson, A.-M.; Martins, S.I.; Stieger, M. Capturing the impact of oral processing behaviour on consumption time and dynamic sensory perception of ice creams differing in hardness. *Food Quality and Preference* **2019**, *78*, 103721.

9. Thomsen, K.; Christophersen, L.; Jensen, P.Ø.; Bjarnsholt, T.; Moser, C.; Høiby, N. Anti-Pseudomonas aeruginosa IgY antibodies promote bacterial opsonization and augment the phagocytic activity of polymorphonuclear neutrophils. *Human vaccines & immunotherapeutics* **2016**, *12*, 1690-1699, <https://doi.org/10.1080/21645515.2016.1145848>.
10. Pereira, E.; van Tilburg, M.; Florean, E.; Guedes, M. Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review. *International immunopharmacology* **2019**, *73*, 293-303, <https://doi.org/10.1016/j.intimp.2019.05.015>.
11. Li, X.; Wang, L.; Zhen, Y.; Li, S.; Xu, Y. Chicken egg yolk antibodies (IgY) as non-antibiotic production enhancers for use in swine production: a review. *Journal of animal science and biotechnology* **2015**, *6*, 1-10, <https://doi.org/10.1186/s40104-015-0038-8>.
12. Müller, S.; Schubert, A.; Zajac, J.; Dyck, T.; Oelkrug, C. IgY antibodies in human nutrition for disease prevention. *Nutrition journal* **2015**, *14*, 1-7, <https://doi.org/10.1186/s12937-015-0067-3>.
13. Garimano, N.; Vergara, L.D.; Kim, A.; Badin, E.E.; Sodero, S.; Bernal, A.M.; Gonzalez, D.; Amaral, M.M.; Lespinard, A.R.; Porporatto, C. Preservation of protective capacity of hyperimmune anti-Stx2 bovine colostrum against enterohemorrhagic Escherichia coli O157: H7 pathogenicity after pasteurization and spray-drying processes. *Journal of dairy science* **2021**, *104*, 5229-5238, <https://doi.org/10.3168/jds.2020-19709>.
14. Miller, A.K.; Williams, S.M. Helicobacter pylori infection causes both protective and deleterious effects in human health and disease. *Genes & Immunity* **2021**, 1-9, <https://doi.org/10.1038/s41435-021-00146-4>.
15. Chua, C.-S.; Yang, K.-C.; Chen, J.-H.; Liu, Y.-H.; Hsu, Y.-H.; Lee, H.-C.; Huang, S.-Y. The efficacy of blueberry and grape seed extract combination on triple therapy for Helicobacter pylori eradication: a randomised controlled trial. *International journal of food sciences and nutrition* **2016**, *67*, 177-183, <https://doi.org/10.3109/09637486.2016.1144716>.
16. Abdi, E.; Latifi-Navid, S.; Abedi Sarvestani, F.; Esmailnejad, M.H. Emerging therapeutic targets for gastric cancer from a host-Helicobacter pylori interaction perspective. *Expert opinion on therapeutic targets* **2021**, 1-15.
17. Mody, R.K.; Hoekstra, R.M.; Scott, M.K.; Dunn, J.; Smith, K.; Tobin-D'Angelo, M.; Shiferaw, B.; Wymore, K.; Clogher, P.; Palmer, A. Risk of Hemolytic Uremic Syndrome Related to Treatment of Escherichia coli O157 Infection with Different Antimicrobial Classes. *Microorganisms* **2021**, *9*, 1997, <https://doi.org/10.3390/microorganisms9091997>.
18. Najdi, S.; Brujeni, G.N.; Sheikhi, N.; Chakhkar, S. Development of anti-Helicobacter pylori immunoglobulins Y (IgYs) in quail. *Iranian journal of veterinary research* **2016**, *17*, 106.
19. Mony, T.J.; Kwon, H.-S.; Won, M.-K.; Kang, Y.-M.; Lee, S.-H.; Kim, S.-Y.; Baek, D.-Y.; Elahi, F. Anti-urease immunoglobulin (IgY) from egg yolk prevents Helicobacter pylori infection in a mouse model. *Food and Agricultural Immunology* **2019**, *30*, 662-676, <https://doi.org/10.1080/09540105.2019.1617251>.
20. Balaraman, H.B.; Rathnasamy, S.K. High selective purification of IgY from quail egg: Process design and quantification of deep eutectic solvent based ultrasound assisted liquid phase microextraction coupled with preparative chromatography. *International Journal of Biological Macromolecules* **2020**, *146*, 253-262, <https://doi.org/10.1016/j.ijbiomac.2019.12.242>.
21. Amro, W.A.; Al-Qaisi, W.; Al-Razem, F. Production and purification of IgY antibodies from chicken egg yolk. *Journal of Genetic Engineering and Biotechnology* **2018**, *16*, 99-103, <https://doi.org/10.1016/j.jgeb.2017.10.003>.
22. Grando, T.H.; Baldissera, M.D.; de Sá, M.F.; do Carmo, G.M.; Porto, B.C.Z.; Aguirre, G.S.; Azevedo, M.I.; de Jesus, F.P.; Santurio, J.M.; Sagrillo, M.R. Avian antibodies (IgY) against Trypanosoma cruzi: Purification and characterization studies. *Journal of immunological methods* **2017**, *449*, 56-61, <https://doi.org/10.1016/j.jim.2017.07.002>.
23. Borges, I.P.; Silva, M.F.; Santiago, F.M.; de Faria, L.S.; Júnior, Á.F.; da Silva, R.J.; Costa, M.S.; de Freitas, V.; Yoneyama, K.A.G.; Ferro, E.A.V.; et al. Antiparasitic effects induced by polyclonal IgY antibodies anti-phospholipase A2 from Bothrops pauloensis venom. *International Journal of Biological Macromolecules* **2018**, *112*, 333-342, <https://doi.org/10.1016/j.ijbiomac.2018.01.178>.
24. Horie, K.; Horie, N.; Abdou, A.; Yang, J.-O.; Yun, S.-S.; Chun, H.-N.; Park, C.-K.; Kim, M.; Hatta, H. Suppressive effect of functional drinking yogurt containing specific egg yolk immunoglobulin on Helicobacter pylori in humans. *Journal of dairy science* **2004**, *87*, 4073-4079, [https://doi.org/10.3168/jds.S0022-0302\(04\)73549-3](https://doi.org/10.3168/jds.S0022-0302(04)73549-3).
25. Nasiri, K.; Nassiri, M.; Tahmoorespur, M.; Haghparast, A.; Zibae, S. Production and characterization of egg yolk antibody (IgY) against recombinant VP8-S2 antigen. *Polish journal of veterinary sciences* **2016**, *19*, <https://doi.org/10.1515/pjvs-2016-0034>.
26. Pauly, D.; Chacana, P.A.; Calzado, E.G.; Brembs, B.; Schade, R. IgY technology: extraction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation. *JoVE (Journal of Visualized Experiments)* **2011**, 3084, <https://doi.org/10.3791/3084>.
27. Herald, T.J.; Aramouni, F.M.; ABU-GHOUSH, M.H. Comparison study of egg yolks and egg alternatives in French vanilla ice cream. *Journal of texture studies* **2008**, *39*, 284-295, <https://doi.org/10.1111/j.1745-4603.2008.00143.x>.

28. Lima, J.G.D.; Brito-Oliveira, T.C.; Pinho, S.C.D. Characterization and evaluation of sensory acceptability of ice creams incorporated with beta-carotene encapsulated in solid lipid microparticles. *Food Science and Technology* **2016**, *36*, 664-671, <https://doi.org/10.1590/1678-457X.13416>.
29. Loibner, M.; Buzina, W.; Viertler, C.; Groelz, D.; Hausleitner, A.; Siaulyte, G.; Kufferath, I.; Kölli, B.; Zatloukal, K. Pathogen Inactivating Properties and Increased Sensitivity in Molecular Diagnostics by PAXgene, a Novel Non-Crosslinking Tissue Fixative. *PLoS One* **2016**, *11*, 0151383, <https://doi.org/10.1371/journal.pone.0151383>.
30. Jaradat, Z.W.; Marquardt, R.R. Studies on the stability of chicken IgY in different sugars, complex carbohydrates and food materials. *Food and Agricultural Immunology* **2000**, *12*, 263-272, <https://doi.org/10.1080/09540100020008137>.