COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Potential allergenicity of chitosan in food contact materials – Additional information and first draft statement.

Note: This is a modified version of the paper circulated to COT Members. The draft statement is the same but commercially confidential information has been removed from the publicly available version of the cover paper.

Background

1. In September 2020, a discussion paper entitled "Allergenicity of chitin and chitosan based BBFCMs (TOX/2020/42)"[1] was presented to the COT, which described the commercial manufacture of chitin and chitosan from the shells of crustaceans. Members noted that additional data characterising the protein content in chitosan and the final BBFCMs would be useful, together with data on migration from and consumption of BBFCMs.

2. Subsequently, in February 2021, a discussion paper on additional information was provided (TOX/2021/10)[2], where the amounts of tropomyosin (Tm) in BBFCMs were estimated, as no consumption or public usage data for chitin or chitosan based BBFCMs were identified in the literature or the National Diet and Nutrition Survey (NDNS) database. The amount of BBFCM that would contain shellfish protein equivalent to the ED01 (an allergen reference dose for crustacean-derived protein, where <1 % of the allergic population may be expected to react) was also estimated.

Additional information

3. Since the COT last considered this topic in February 2021, additional information has become available from Fera Science Ltd., and a business developing chitosan-based packaging.

4. Fera currently has a PhD research project on chitosan films, although this work does not include safety and migration testing. It was noted that research on chitosan films is more focussed around enhancing antimicrobial properties than consumer safety assessments. No research papers specifically looking at migration in terms of food safety were identified, though there are papers that include

[1] https://cot.food.gov.uk/sites/default/files/2020-09/TOX-20-42%20Chitosan%20%26%20chitin%20BBFCMs.pdf

[2] https://cot.food.gov.uk/sites/default/files/2021-01/TOX-2021-03%20Additional%20Information%20on%20Allergenicity%20of%20Chitin%20and%20Chitosan%20Based%20BBFCMs.pdf

antimicrobial compounds in chitosan matrices (films and nanoparticles) for 'active' packaging for increasing shelf-life. It was also noted that using examples of migration for other allergens would have a high level of uncertainty, as any migration will be impacted by various factors (i.e. contact conditions, polarity and diffusion characteristics of the film, peptide polarity, partition coefficient, and diffusion within the film and foodstuff).

5. Fera is conducting the contaminants testing on BBFCMs as part of an EU funded project, and will share the results with the FSA.

6.

Exposure assessment

7. In their previous discussions, the Committee noted that it would be useful to have an indication or estimation of total exposures to allergenic proteins in BBFCMs, for example the upper bound levels of ingestion, or range of amounts of BBFCMs in contact with different foods. However, this has not been conducted at present due to the uncertainties involved without analytical data (such as those uncertainties noted by Fera above), and the Exposure team is pending additional further allergen data which are becoming available around mid-November, as noted.

Draft statement

8. A statement setting out the currently available information on chitosan is attached and identifying the need for additional information is attached Annex A to this paper.

Questions for the Committee:

9. Do Members have any comments on:

i) The new information provided?
ii) The possibilities for exposure assessment?
iii) The structure and content of the draft statement?
iv) Any other comments?

Secretariat
August 2021

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on potential allergenicity of chitosan in food contact materials

Background

1. The use of fossil-based plastics has been associated with adverse environmental impacts. Consequently, there is increasing interest in reducing the amount of conventional plastic used for packaging. As a result of government initiatives around the world, and pressure from environmentally-aware consumers, recent years have seen a major global increase in the development and use of biobased food contact materials (BBFCMs). Biobased materials are defined as being derived, directly or indirectly, from a renewable source of living matter (Bradley, 2010).

2. Some BBFCMs contain chitosan, which is a biodegradable polysaccharide derived from chitin (Figure 1). Chitosan is an “active” agent as it has antimicrobial and antioxidant properties (Vasile, 2018). These properties make it ideal for extending the shelf-life of packaged foods.

3. Zhang *et al.* (2011) have reviewed various studies in which chitosan exhibits antifungal activity. Chitosan inhibits fungal growth including *Fusarium* spp., and has been shown to reduce mycotoxin production in treated crops (Zachetti *et al.,* 2019). Chitosan can also behave as an adsorbent to remove various mycotoxins, in animal feed for example (Pirouz *et al.,* 2020).

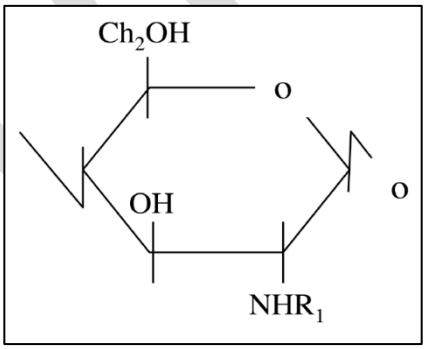

Figure 1: Chemical structures of chitin (R_1 = $COCH_3$) and chitosan (R_1 = H). Chitin is a high molecular weight β(1,4)-linked homopolymer of N-acetylglucosamine. *In situ*, chitin is linked to other structural components, such as protein and glucan, to form a protein-chitin matrix (Romano *et al.*, 2007).

4. One limitation of using chitin on a large commercial scale is its insolubility in water. Therefore, derivates have been produced from chitin that are more water-soluble, of which chitosan is the most important commercially. Chitosan exists naturally in only a few species of fungi such as zygomycetes (Muzarelli *et al.*, 1994).

5. Presently, the main commercial source of chitosan is from chitin obtained from waste streams from the marine fishery industry, i.e. the shells of crustaceans. However, the recent increase in demand for chitosan in the global market has drawn attention to other possible sources of chitosan independent of marine fishery waste. These are the cell walls of fungi and the exoskeletons of insects. Their availability is less limited by geography and season.

6. There are some concerns regarding the potential allergenicity of chitin and chitosan, as the biological sources that these materials are derived from contain allergenic proteins, in particular tropomyosin (Tm). The FSA has also received a number of queries about the presence of chitosan in packaging and food films, and chitosan-based drinking straws.

UK incidents

7. Although no UK incidents have been raised formally, there is one report of a potential reaction to the use of a chitosan-based straw in a pub which was reported to a local authority. The local authority carried out an investigation with the supplier of the chitosan-based straws. Whilst there was some uncertainty, the circumstances in this situation made it difficult to rule out cross-contamination from the meal that the individual also consumed on the premises. The individual who suffered the allergic reaction did have a seafood allergy but did not disclose this to the pub.

Sources of chitosan

Chitosan derived from crustaceans

8. Hahn *et al.* (2020) provide an overview of the industrial process for chitin purification and chitosan production from the shells of crustacean. This process involves deproteination to remove proteins such as Tm from chitin. Purified chitin is then deacetylated to form chitosan. The deproteination and deacetylation steps are described below in paragraphs 9 and 11, respectively.

9. Deproteination can be conducted using chemical methods, which are well established for the commercial preparation of chitin (EC, 2018). Sodium hydroxide (NaOH) is the preferential reagent for chemical methods, and is used to solubilise the proteins present; it is applied at concentrations ranging from 0.125 to 5.0 M, at varying temperature (up to 160 °C) and treatment duration (from few minutes up to few days) (Younes & Rinaudo, 2015). Alternatively, biological (enzymatic) methods can be used for the deproteination of chitin.

Enzymatic methods use whole cell microorganisms (Xu *et al.*, 2008) or purified enzymes (De Holanda & Netto, 2006; Synowiecki & Al-Khateeb, 2000). However, to date, use of the enzymatic method for deproteination has been limited to laboratory scale studies (Gadgey & Bahekar, 2017).

10. The table in Annex 2 shows the degree of deproteination (DP) for chitin extracted from marine resources which has been achieved in literature studies. This shows some variation for the conditions of deproteination for chitin preparations using enzymatic and chemical methods, and for the DP obtained which ranges from 40 to >99 % across the studies reviewed (additional details in Annex 2). However, according to Berezina & Hubert (2020), “no completely effective method for the determination of this (chitin) purity exists… due to the high insolubility of the polymer”. A modified spectrophotometric method according to Lowry *et al.* (1951) is often used for quantification of protein in chitin samples (e.g. Bajaj *et al.*, 2011).

11. After deproteination, chitin is converted to chitosan by removing the acetyl groups (COCH3). This process of deacetylation is done though enzymatic (biological) or chemical methods. Chemical methods are used more extensively than enzymatic methods for commercial preparation of chitosan because of lower cost and suitability for mass production (No *et al.*, 1995).

12. The extent of N-deacetylation throughout the polymer is almost never complete as some acetamide groups usually remain (Abdulkarim *et al.*, 2013). This gives rise to different degrees of deacetylation (DD). The DD is generally defined as the glucosamine/N-acetyl glucosamine ratio. When the percentage of N-acetyl glucosamine > glucosamine, the polymer is called chitin. Conversely, when the percentage of glucosamine > N-acetyl glucosamine, the compound is called chitosan (Viarsagh *et al.*, 2010). Chitosan has also been defined as chitin that is sufficiently deacetylated to form soluble amine salts (NTP, 2017).

13. Solubility of chitosan in aqueous, acidic media occurs when deacetylation of chitin reaches approximately 50 % (Rinaudo, 2006), though in addition to the DD, chitosan solubility is also dependent on the molecular weight and the distribution of the remaining acetyl groups on the polymer (Kubota & Eguchi, 1997). Experiments conducted by Ottsy *et al.* (1996) show compositional heterogeneity in the chitosans, with chitin-like acid insoluble fractions with acetylated units between 88-95 %, and fractions with acetylated units from 20-52 %. The DD influences both chemical (e.g. solubility) and biological (e.g. bioavailability and biodegradability) properties of chitosan (Benhabiles *et al.* 2012; Park & Kim, 2010).

14. The high density of positive charges that are left on the amino groups after deacetylation make chitosan more water-soluble and allows it to interact with negatively charged substances such as proteins, fatty acids, bile acids, and phospholipids. These interactions give rise to antimicrobial, antioxidant, and fat-binding properties of chitosan, leading to potential applications in the food industry, e.g. preservative, packaging additive, and dietary supplement (Sethulekshmi, 2014).

Chitosan derived from fungi

15. Chitin/chitosan derived from fungi is devoid of Tm (Nwe & Stevens, 2002). Commercialisation of non-animal ('vegan') chitosan is at an early stage, with few attempts to produce at large scale and a limited number of firms selling the products (EC, 2018).

16. Although not all fungi contain chitin, it is distributed in various fungal phyla such as Ascomycota, Basidiomycota, and Zygomycota. The most investigated species for chitosan production include *Aspergillus niger* (Ascomycota), *Lentinus edodes* (Basidiomycota), *Absidia coerulea, and Absidia glauca* (Zygomycota), and *Rhizophus oryzae* and *Mucor rouxii* (Mucoromycota). However as noted above, the production of fungal chitosan has not yet been scaled up to the industrial level. (Hahn *et al.* 2020).

17. In 2010, the EFSA panel on Dietetic Products, Nutrition and Allergies (NDA) assessed the safety of chitin-glucan derived from *Aspergillus niger* as a novel food ingredient (EFSA, 2010). This chitin-glucan was derived through a fermentation process, and did not contain shellfish protein. The product assessed by EFSA was called "KiOnutrime-CG™", composed of >90 % chitin-glucan (a structure that combines chitin and beta (1,3) glucan) and ≤ 6 % protein, and was intended to provide an intake of 2 to 5 g chitin-glucan/day. The Panel reviewed a report showing no observed adverse effects at the highest dose administered (about 6.6 g/kg bw) in a 13-week rat study (TNO, 2009). Because this dose is approximately 80-fold higher than the maximum intended level of intake for humans on a g/kg bw basis, the Panel concluded that KiOnutrime-CG™ was safe as a food ingredient at the proposed conditions of use and at the proposed intake levels. The Panel assessed the risk of allergenicity on the basis of some allergenic enzymes that are synthesised by *A. niger* such as beta-xylosidase. The Panel concluded that "an allergenic risk cannot be ruled out, but is expected not to be higher than the consumption of other *A. niger* derived products".

Chitosan derived from insects

18. Insects are a viable alternative source of chitin, but they have not been exploited in the past due to limited availability. However, insect farms are being developed to provide insects for animal and human nutrition (Ortiz *et al.*, 2016; Hahn *et al.*, 2020).

19. The exoskeleton of insects, which is shed from the body during metamorphosis, is rich in chitin. To extract the chitin, a purification process is used to remove proteins, lipids, minerals, pigments, and catechols contained therein (Mohammed *et al.*, 2013). In their review, Hahn *et al.* (2020) identified 52 papers reporting chitin purification and chitosan production from 58 insect species. However, Hahn *et al.* note that although "multiple studies have described the extraction of insect-based chitin and its subsequent conversion to chitosan… discrete and quantitative values regarding yield and degree of purification are missing".

20. Reports on adverse reactions after eating insects are scarce and only two population studies were identified in the literature which report on the prevalence of food allergy to insects. A review of insect (food) allergy and allergens conducted by Gier & Verhoeckx (2018) showed that various insect allergens have been identified, including Tm.

21. Taylor & Wang (2018) investigated the prevalence of allergic reactions caused by consuming edible insects. The investigation was conducted in the North Eastern (or the Isan region) of Thailand, in an area where insect consumption (or entomophagy) is a common practice. Information concerning insect consumption and allergic reactions were gathered from multiple sources in four locations: Nongki, Nang Rong, Nong Bun Mak, and Nakhon Ratchasima. The survey included questions about eating habits in relation to insects, other known food allergies, and presented a list of symptoms the participants may have experienced. The prevalence of allergic reactions caused by consuming edible insects was much higher than expected across the 2,500 respondents. In the Isan region, approximately 7.4 % of people experienced an adverse reaction indicative of an edible-insect allergy, and 14.7 % of people experienced multiple adverse reactions “indicative” of an edible-insect allergy. Furthermore, approximately 46.2 % of people that already suffer from a known food-based allergy also experienced symptoms indicative of an allergic reaction after insect consumption. According to the study authors, “the most common symptoms appear to be gastrointestinal (diarrhoea and vomiting)”. The study authors concluded that “the allergy aspect of entomophagy is a serious issue and has the potential to adversely affect the future of entomophagy, especially in introducing the concept to western cultures”.

22. Barennes *et al.* (2015) assessed the prevalence of food allergy to insects amongst insect-eaters. In this survey, eight teams (which included medical physicians) collected data to address socioeconomic characteristics of the consumers, types of insects consumed, frequency of consumption and reports of side effects. This study was conducted in Laos, and included 1,059 subjects that had previously eaten insects, 81 of whom (7.6 %) reported “allergy problems after eating insects”. Of these 81 subjects, 38 reported that allergy problems were “mostly with grasshoppers or stink bugs”. None of the subjects reported severe anaphylaxis. In this survey, it was not possible to know how much the consumption of edible insects represents the daily diet of the population, or provide detail on the way insects were harvested. It does not mention any clinical confirmation of allergy problems.

23. Clinical measurements of allergy do not seem to have been verified in the two available surveys. Additionally, the work of Broekman *et al.* (2017) demonstrates the possibility of *de novo* sensitisation to allergens in mealworm.

Market uses of chitin & chitosan

24. Chitosan has applications in various fields such as tissue engineering and biomedicine due to its low cost, biocompatibility, lack of toxicity, and biodegradability (Madhumathi *et al.*, 2009; Konovalova *et al.*, 2017).

25. Chitosan is widely used in as a food additive and functional ingredient in foods sold in Italy, Finland, Korea and Japan (Peter, 1997; Singla & Chawla, 2001). The Norwegian company "Norwegian Chitosan AS" trades chitosan (Kitoflokk™ and Norlife) for several applications, including food and beverages[3] (Ferreira *et al.* 2016).

26. Chitosan and chitin have not been officially classified as GRAS (generally recognised as safe) by the US Food and Drug Administration (US FDA). Rather, some biotechnology companies have notified the US FDA of their view that the use of chitosan and chitin in specific food applications is GRAS. For example, KitoZyme views the use of chitosan (derived from *Aspergillus niger*) in alcoholic beverage production (with chitosan being removed from the beverages post-treatment, using physical separation processes) as GRAS. In their correspondence to KitoZyme, the US FDA (2011) concluded that: "based on the information provided by KitoZyme, as well as other information available to FDA, the agency has no questions at this time regarding KitoZyme's conclusion that chitosan from *A. niger* is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of chitosan".

27. Presently, the following notices appear on the FDA website[4] for chitosan and chitin:

- *Agaricus bisporus*-derived chitosan, for use as "an antimicrobial at levels ranging from 0.015 g to 0.15 g per 100 g of food in baked goods and mixes, alcoholic cocktail drinks, sports drinks, soft drinks, flavored waters, ready-to-drink coffee and tea, cheeses, imitation cheese, yogurt, condiments and relishes, confections and frostings, bars (meal replacement, snack), pastas, gravies and sauces, jams and jellies, processed fruit and fruit juices, vegetable juices and purees, soups, dressings and spreads, puddings and fillings, sugar substitutes, meat analogs, and sweet sauces, syrups and toppings", by Chinova Bioworks Inc. (GRS no. 997).
- *A. niger*-derived chitosan, used as a "secondary direct food ingredient in alcoholic beverage production at levels between 10 and 500 grams per hectoliter (100 liters)", by KitoZyme (GRN no. 397).
- *A. niger*-derived chitin, for "use in microbial stabilization, removal of contaminants, and/or clarification in alcoholic beverage production at levels between 10 and 500 grams per hectolitre", by KitoZyme (GRN no. 412).
- Shrimp-derived chitosan, for "use in foods generally including meat and poultry, for multiple technical effects", by Primex (GRN no. 443).
- Shrimp-derived chitosan, for use as an "ingredient in food including meat and poultry products", by Primex (GRN no. 170).
- Shrimp-derived chitosan, for "use in foods in general for multiple technical effects in accordance with good manufacturing practice", by Primex (GRN no. 73).

28. A submission by Primex to the US FDA in 2012 (GRAS Notice No. 443) contains a dossier which includes some approaches to protein measurement and

[3] http://www.chitosan.no/?page_id=1266

[4] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=advanced&search=%C2%A4%C2%A4chitosan%20chitin%C2%A4

analytical data for the ED01 and corresponding analysis[5]. This dossier was considered by the COT in discussion paper TOX/2021/03, where the Committee noted that the chitosan used in this submission appeared to be highly controlled in terms of its production, and whilst its specification may be unlike that of other chitosan products, it nevertheless provides a standard to be achieved and possibly put forward.

29. Chitosan is sold online as a dietary supplement, where manufacturer-recommended daily consumption of chitosan is, for example, 2.4 g[6] and 3 g[7]. In 2011, when reviewing a proposed health claim, the EFSA NDA Panel concluded, that "a cause and effect relationship has been established between the consumption of chitosan and maintenance of normal blood LDL-cholesterol concentrations", and "considers that in order to obtain this effect in adults, 3 g of chitosan should be consumed daily" (EFSA, 2011).

30. Chitosan is considered to be hemostatic due to its cationic nature (NTP, 2017), which supports its use in wound dressings. Wound dressings manufactured from shellfish-derived chitosan are available for clinical use (Wedmore *et al.*, 2006). Waibel *et al.* (2011) investigated the safety of these "HemCon®" bandages, that were introduced in 2005 for US soldiers. Patients who reported shellfish allergy were recruited. Initial assessment included a detailed history, IgE skin prick testing (SPT), and serum testing to shellfish allergens. Participants who demonstrated specific shellfish IgE underwent a bandage challenge. It was reported that of the nineteen participants who were enrolled, 10 completed the study. Seven (70 %) were male and the average age was 44.8 + 10 years. Nine (90 %) reported a shrimp allergy history and five (50 %) reported multiple shellfish allergies. All participants completing the study had positive SPT and serum IgE testing to at least one shellfish; eight (80 %) had shrimp positive SPT and ten (100 %) demonstrated shrimp-specific IgE. No participant had a positive SPT to chitosan powder or experienced an adverse reaction during bandage challenges. No protein bands were visualised during gel electrophoresis analysis of chitosan powder. The study authors concluded that all participants tolerated the HemCon bandage without reaction. This is the first study demonstrating the safety of this bandage in shellfish allergic subjects. The US FDA approved the HemCon bandage for use as a dressing for local management of bleeding wounds in 2008[8].

[5] http://wayback.archive-it.org/7993/20171031043636/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM337459.pdf

[6] https://gb.pipingrock.com/weight-loss-support/ultra-lipo-chitosan-per-serving-800-mg-240-quick-release-capsules-2313?prd=129738a3&prisp=1&gclid=Cj0KCQjwvO2IBhCzARIsALw3ASoe7oBTJX4IgTxS12EwS9vw_XoL_EyU-VHvfsydYJguU32ZEe4bwroaAhk_EALw_wcB

[7] https://www.amazon.co.uk/Best-Naturals-Chitosan-500-Tablets/dp/B00KPXAC28/ref=asc_df_B00KPXAC28/?tag=googshopuk-21&linkCode=df0&hvadid=310665675964&hvpos=&hvnetw=g&hvrand=22302337432427496&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=1007266&hvtargid=pla-562291778125&psc=1

[8] https://www.accessdata.fda.gov/cdrh_docs/pdf8/K080818.pdf

31. The UK Medicines and Healthcare products Regulatory Agency (MHRA) is aware of chitin and chitosan being used in medical devices, but is "not aware of a safety issue investigated by the MHRA related to this material that has come to light since receiving market authorisation" (FSA pers. comm.).

Chitosan BBFCMs in development

32. Recent research has addressed the development of composite films for food packaging with additional or enhanced properties such as antimicrobial and antioxidant activities. These "smart materials" have included the use of chitin or chitosan in their composition. Research on food packaging based on chitin films is less common than for chitosan films, as chitin is less soluble.

33. The utilisation of chitosan in food packaging is either in the form of flexible films or of coatings (Priyadarshi & Rhim, 2020). A "film" is preformed separately and wrapped onto a food surface later, whereas a "coating" is a thin layer formed directly onto the food's surface. Films may be described as edible or inedible, whereas coatings are almost always edible since they form a layer directly on the top surface of the food (Priyadarshi & Rhim, 2020).

34. The most commonly investigated polymers for making edible films/coatings are polysaccharides (chitin/chitosan, cellulose, starch, pectin, seaweed extracts, gums, pullulan), proteins (gelatin, soy protein, zein, wheat gluten, myofibrillar protein, milk protein), and lipids (synthetic/natural waxes, vegetable/animal oils and fats, essential oils and extracts, resins). The properties of the film/coating, such as cohesion, adhesion and durability, depend on the composition of material, coating method and drying method (Jeevahan & Chandrasekaran, 2019).

35. Chitosan films can be divided into edible films or coatings (< 30 μm thickness), for application directly on food, and films (>30 μm thickness) (Van den Broek *et al.*, 2015). However, with the advancement of nanotechnology, new concepts such as nano-coatings, which consist of nanoscale layers (less than 100 nm) built-up onto surfaces, are being explored (Vasile, 2018; Müller *et al.*, 2017). In their review of "nanoedible films" for food packaging, Jeevahan & Chandrasekaran (2019) noted that production of edible films and coatings is still largely at the laboratory level and is not yet expanded to industrial level due to their high cost of production.

36. Flexible chitosan films are usually prepared by the solvent casting method in which chitosan is dissolved in suitable solvents, in most cases slightly acidified water, and is poured on a flat surface to allow the solvent to evaporate (Kim *et al.*, 2006). Direct application of chitosan formulations onto food surfaces can be attained by spraying or dipping (Tharanathan, 2003). Because chitosan films and coatings are created from diluted acid solutions, they can remain water sensitive, or even water soluble, which limits their range of applications.

37. A manufacturer of chitosan-based food packaging has indicated that it sees value in developing packaging for a broad range of food applications (including meat, fish, cereals, bakery products, fruits, and vegetables). This

manufacturer has indicated that average levels of total protein in its prototype purified chitin is 0.1 %, where levels of Tm are < limit of quantification (of 20 ppb), and generally ≤ the limit of detection (LOD) of 1 ppb.

38. At a research stage, it has been proposed that "the edible and biodegradable chitosan-based films used for food packaging can also be consumed along with packaged food" (Yadav *et al.* 2019). It is suggested such films could appear in vacuum-packaged processed meat (Ouattara *et al.*, 2000), cheese (Fajardo *et al.*, 2010), and other foods such as vegetables, fruits, grains, and fish (Sinha *et al.*, 2012). Chitosan can also be used as an inhibitor of browning in juices (Abdelmalek *et al.*, 2017), and an antioxidant in sausages (Arslan & Soyer, 2018).

39. Modifying chitosan by the addition of a metal enhances its antimicrobial activity compared to native chitosan (Du *et al.*, 2009). For example, the antimicrobial activity of chitosan- Zn^{+} (zinc) and chitosan-Ag^{+} (silver) is higher than native chitosan (Zhang *et al.*, 2016; Wei *et al.*, 2009). Subsequently, some of the chitosan-based BBFCMs are nanoengineered to contain metal ions. For example, Yin *et al.* (2018) prepared carboxymethyl chitosan/poly(vinyl alcohol)/Cu (copper)blend film for packaging application..

40. The n-CHITOPACK project coordinated by Mavi, Italy was initiated with the objective of developing new chitin-based food packaging material by utilising chitin nanofibrils with other natural polymers (Morganti, 2013).

41. Wu *et al.* (2019) developed a novel intelligent film by immobilizing 1 %, 3 % and 5 % black rice bran anthocyanins (BACNs) into oxidized-chitin nanocrystals (O-ChNCs)/ chitosan (CS) matrix. The ultraviolet-visible spectrum of BACNs solutions showed colour variations from red to greyish green in a range of pH 2.0–12.0. The study authors concluded that the results showed that the CS/OChNCs/BACNs (COB) films containing 3 % of BACNs (COB-3) were able to monitor the spoiling of fish and shrimp by visible colour changes. Therefore, the authors noted that these COB-3 films could be used as an intelligent food packaging for monitoring animal-based protein food spoilage.

42. Sahraee *et al.* (2017a) developed gelatin-based bionanocomposite films (GNCFs) containing 0, 1, 3, and 5 % zinc oxide nanoparticles (N-ZnO) and/or 0, 3, 5, and 10 % chitin nanofibers. Simultaneous incorporation of chitin and ZnO nanoparticles in the GNCFs had the interactive effect on improving the physicochemical and antimicrobial properties of GNCFs. Sahraee *et al.* concluded that the GNCFs "showed better physical and antifungal properties than net gelatin films and can be applied for increasing storage life of packaged foods".

43. Panariello *et al.* (2019) treated cellulose-based board packaging with chitosan and chitin nanofibrils in varying ratios. Trials performed with packaged food demonstrated that chitin and chitosan were effective in reducing the microbial growth, thus allowing an increase of food shelf life. The study authors concluded that "the results confirmed that it will be reasonably possible to

increase food safeness and to waste less food thanks to the use of a fully renewable and biodegradable packaging".

44. Chitin is present in some BBFCMs as nanofibers (Ifuku & Saimoto, 2012) or "nano-whiskers" (Zeng *et al.*, 2012). Chitin nano-whiskers are the crystalline part of fibers, often termed nanocrystals that are devoid of amorphous regions. They are shorter and have more defined dimensions. The dimensions of chitin nano-whiskers, when extracted from shrimp shells using hydrochloric acid hydrolysis, are 110-975 nm (length) and 5-74 nm (width) across reviewed studies (Mincea *et al.*, 2012). Incorporation of chitin nano-whiskers into starch-based films has been shown to improve the film's mechanical and barrier properties (Qin *et al.*, 2016), and may be regarded as a "passive" material.

45. Regarding chitin nano-whiskers, and nanoparticles more generally, migration studies are scarce. This is due to the difficulties in characterising nanoparticles in composites, and the lack of methods for qualitative and quantitative analysis (Han *et al.*, 2011). Indeed, the use of nanoparticles in the development of food packaging materials is still a novel field (Huang *et al.*, 2015). Food matrices are complex, and one single technique is not enough to provide all information, thus extra fractionation procedures and combined detection methodologies are often needed.

UK legislative position

46. In retained European legislation, all materials and articles intended for contact with food must meet the requirements of the Framework Regulation (EC) No 1935/2004. The principle underlying this Regulation is detailed in Article 3 which states: "materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: a) endanger human health; b) bring about an unacceptable change in the composition of the food; c) bring about a deterioration in the organoleptic characteristics thereof."

47. With regards to necessary labelling (and potential exposure to allergens) Article 15 of retained Regulation (EC) No 1935/2004 states that 'special instructions (are) to be observed for safe and appropriate use'. This labelling information may need to be provided on the packaging, or as a standalone warning should the item be sold loosely. If the item was marketed as edible, other labelling requirements come into play to comply with food law and the Materials and Articles in Contact with Food Regulations 2012 as amended.

48. In Directive 90/128/EEC, the European Commission published overall migration limits (OMLs) and specific migration limits (SMLs) which apply to plastic food contact materials (EC, 1990). This Directive has been superseded by Regulation 10/2011 (EC, 2011), in which the majority of migratory limit values remain unchanged. Whilst there are no specific migration limits for BBFCMs, industry can refer to legislation that may be pertinent (the same holds true for other materials lacking specific legislation). Furthermore, the Plastics Directive stipulates a generic

migration limit of 10 mg per square decimetre of surface area of material (10 mg/dm^2) which is applicable under these circumstances. The applicability of FCM legislation depends on the BBFCM's intended use and how it is marketed. If the BBFCM is intended purely for containment purposes and is inedible, it is not food and comes under FCM legislation.

49. The EU considers that an edible film is a special active part of the food and, seen from a legal point of view, it is to be regarded as a foodstuff, along with the food packed in the film, having to fulfil the general requirements for food (Fabec *et al.*, 2000). Subsequently, the presence of a known allergen on an edible film or coating on a food must be clearly stated in the label (Campos *et al.*, 2011). Due to hygienic reasons, it is anticipated that food products in edible films need to have an outer package, otherwise the film should not be eaten (Fabec *et al.*, 2000).

ADME & **toxicity of chitin and chitosan**

50. Results from Chae *et al.* (2005) indicate that absorption of chitosan from the gastrointestinal tract following oral exposure in rats is inversely related to its molecular weight: oral gavage administration of chitosan with molecular weights of 3.8, 7.5, 13, 22, or 230 kDa resulted in maximum plasma chitosan concentrations (Cmax) of 20.23, 9.30, 5.86, 4.32, or <0.5 µg/mL, respectively. Degradation of chitosan in vertebrates is thought to occur predominantly by lysozymes and bacterial enzymes in the colon (Kean & Thanou, 2010). The rate of biodegradation of chitosan *in vivo* is dependent on the DD (Yang *et al.*, 2007).

51. Chitooligosaccharides (COS), having a molecular weight of approximately 10 kDa or less, are the depolymerised products of chitin or chitosan, and can be produced through chemical hydrolysis or enzymatic methods (Xia *et al.*, 2010). Enzymatic methods can use various enzymes including chitinase and chitosanase (Klinkesorn, 2013). Various non-specific enzymes can also break down chitosan including lysozymes, cellulases and lipases, which help with its biodegradation in nature (Raafat & Sahl, 2009). Chitosan with a molecular weight of ≤ 16KDa is considered a COS (Rajoka *et al.*, 2020). COS are water-soluble (Qin *et al.*, 2006), and have antioxidative, anti-inflammatory, and antibacterial effects (Huang *et al.*, 2016). However, COS have been observed to irritate intestinal epithelial mucosal tissues, stimulating them to hyperproduce mucin (Deters *et al.*, 2008).

52. Studies designed to evaluate the effectiveness of chitosan as an oral weight-loss supplement over 12 days suggest that it is well tolerated in men and women at 4.5 g chitosan per day (Gades & Stern 2003, 2005). Data collection sheets for the volunteers did not appear to have a space for recording any adverse effects, but one of the 15 male participants reported "vomiting after a meal during the supplement period" (Gades & Stern 2003). Additionally, in a study involving 65 men and women, consumption of chitosan tablets (6.75 grams of chitosan daily for eight weeks), was "found to be safe", though common transient gastrointestinal symptoms were reported (loose faeces, constipation,

abdominal pain, repeated flatulence, abdominal bloating, and abdominal rumbling) (Tapola *et al.*, 2008).

Hazard identification

53. Incomplete deproteination of chitin may lead to the presence of allergenic proteins in the final material such as Tm. Tm is a muscle protein which, together with myosin and actin, is involved in muscle contraction. However, many isoforms of Tm exist and Tm is also present in non-muscle cell types (Reese *et al.*, 1999). Tm is present in all species of vertebrates and invertebrates. However, only the Tm found in invertebrates such as crustaceans, arachnids, insects, and molluscs is associated with allergic reactions in humans (Lehrer *et al.*, 2003; Lopata *et al.*, 2010; Reese *et al.*, 1999). Tm is considered to be the major allergen in seafood (Faber *et al.,* 2016). Tm can cause allergic reactions in sensitised individuals, and different IgE-binding B- and T-cell epitopes in Tm have been described (Subba *et al.*, 1998).

54. Tm is a heat-stable allergen (Daul *et al.*, 1994). It is also an “acidic” protein with an isoelectric point (pI) value of 4.5 (Reese *et al.*, 1999), and thus its conformational structure has some resistance to acidic conditions. Due to these characteristics, Tm can be present in processed foods (Hoffman *et al.*, 1981; Lopata and Lehrer, 2010; Nagpal *et al.*, 1989; Reese *et al.*, 1999).

55. Some researchers do not recommend the use of chitosan in the diet of individuals who are allergic to crustaceans (Ylitalo *et al.*, 2002). The most widely accepted allergen reference doses for crustacean-derived protein are ED01 (where <1 % of the allergic population may be expected to react) at 26.2 mg of shrimp protein, and ED05 at 280 mg of shrimp protein (Remington *et al.*, 2020). These reference values are derived from human food challenge data, and represent acute intake levels of crustacean-derived protein that are predicted to provoke an objective reaction in no more than 1 and 5 % (respectively) of at-risk individuals, who show a minimal allergic response upon challenge. An allergenic reference dose for Tm alone was not identified in the literature.

56. In their review of the safety of chitosan, Ylitalo *et al.* (2002) noted that chitosan is not specifically hydrolysed by digestive enzymes, but limited digestion of chitosan may occur due to bacterial flora and the unspecific activities of some digestive enzymes such as amylase and lipase. In addition, several mammalian chitinases and chitinase-like genes have been identified in humans (Boot *et al.*, 2001). Boot *et al.* (2005) discussed the possibility that gastrointestinal chitinases might have a dual function, in immune defence and in food digestion.

57. Since approximately 1 % of the world population is allergic to shrimp (e.g. Sicherer *et al.*, 2004; Castillo *et al.*, 1994), the probability of a reaction in the population exposed to the ED01 is therefore 1 % of 1 %. Despite this low percentage, widespread usage may affect a significant number of people, thus appropriate risk management measures are important, such as labelling to declare allergenic source(s), and consumer awareness unless exemptions are

obtained. For edible packaging, these aspects should be covered by existing legislation.

58. The Committee considered the ED01 was an adequate protection goal, given the potential for increased human exposure to the allergen if it were to be present in food packaging. It was agreed that the choice of benchmark (e.g. ED01) is a risk management decision or benchmark. Due to the large amount of data used for dose distribution modelling, accurate estimates below ED01 are not feasible.

59. The Committee considered that in order to assess whether FCM posed no health risk in practice (if consumption was below the ED01), it would be necessary to know the effects of processing on the levels of allergens in the final material, which may then migrate into food, as was the case for other allergens.

60. Rahman *et al.* (2010) analysed the allergenic proteins in Black Tiger shrimp using peptide mass finger printing and peptide fragment fingerprinting methods. Their study found the presence of Pen m 2 protein with arginine kinase activity, Tm, and myosin light chain (MLC) (Rahman *et al.*, 2010). Subsequently, Nguyen (2012) noted “a need to examine the presence of these proteins in chitin and chitosan” which “could cause allergic reactions”.

61. Nguyen (2012) used immunoblotting techniques to investigate the presence of Tm in protein extracts of shrimp, and also technical samples of chitin and chitosan. The study could not isolate or quantify the residual proteins from the samples of chitin and chitosan and thus measurements of Tm concentrations in chitin and chitosan samples were not reported.

62. Nguyen (2012) noted that “many methods have been tried to isolate and identify the residual proteins in chitin and chitosan samples. However, they were not successful. There are many possibilities that make it hard to separate proteins from chitin and chitosan sample. Firstly, the residual proteins must be combined tightly in the structure of the chitin and chitosan powder, so they can survive after treatment with high acidic and alkaline solutions during their extraction. The second reason could be related to the solubility of chitin and chitosan. Chitin cannot dissolve in normal solution (Pillai *et al.*, 2009; Sannan *et al.*, 1975); if strong chemicals were used to dissolve chitin, harsh environment will break down the residual proteins. On the other hand, chitosan can dissolve in light acidic condition; however this solution is too viscous to run through the filter to separate proteins. Chitosan solutions are also very sticky that they cannot be separated by SDS-PAGE. Another reason could be because most of the proteins remaining in the chitin and chitosan were degraded and broken down into small fragments during extraction from the shrimp shell, so they cannot be separated by SDS-PAGE and Western blot”.

63. Overall, the studies conducted by Nguyen (2012) demonstrated the presence of Tm protein in the chitin and chitosan samples, where the antibodies were able to interact with Tm. Subsequently, Nguyen (2012) noted that “special care should be taken when using chitin and chitosan in food or medical preparations. Warning statements should state clearly the presence of Tm in

products derived from chitin or chitosan, especially when the consumers are sensitised to crustaceans".

Hazard characterisation

Exposure assessment

64. No measurements of the amount of shellfish protein in BBFCMs were found in the literature. No consumption or public usage data for chitin or chitosan based BBFCMs were identified in the literature or the National Diet and Nutrition Survey (NDNS) database. Therefore, to assess the risk of allergenicity with respect to the ED01 of 26.2 mg for shrimp protein, a preliminary estimation of the % (wt) shellfish protein in BBFCMs was conducted for both edible BBCFCMs (films or coatings which are intended to be consumed with the food), as well as inedible BBFCMs (assuming that all shellfish protein present could be consumed due to 100 % migration into food) (see Annex 3 for the detailed calculations).

65. For non-edible BBFCM films containing chitosan and/or chitin nano-whiskers, it was estimated that the amount of BBFCM that would contain shellfish protein equivalent to the ED01 was 2-52 grams across the studies reviewed (Annex 3, Table 1). For edible BBFCM films and coatings containing chitosan, it was estimated that the consumption of BBFCM to reach ED01 was 131-262 grams across the studies reviewed (Annex 3, Table 2).

66. The Committee noted that it would be useful to have an indication or estimation of total exposures to allergenic proteins in BBFCMs, for example the upper bound levels of ingestion, or range of amounts of BBFCMs in contact with different foods. However, due to the uncertainties involved, this is pending further measurement data which is becoming available.

67. Taking into account the available data, it is not currently possible to undertake a reliable exposure assessment.

Immunogenicity of chitin & chitosan

68. Komi *et al.* (2019) consider that chitin and chitosan are potential targets for recognition by the mammalian immune system since mammalians lack such biopolymers naturally. Thus, Patel & Goyal (2017) note in their review that "caution should be exercised while using it for food and therapeutic purposes".

69. Upon exposure, chitin can be recognised by mammalian chitinases that bind and degrade chitin, and chitinase-like proteins which also bind chitin but are catalytically inactive (Funkhouser & Aronson, 2007). Furthermore, both chitin and chitosan particles are readily phagocytosed, supporting a role for recognition via specific receptor(s) mediating phagocytosis, though the receptor(s) remain to be determined (Bueter *et al.,* 2011).

70. Chitin and chitosan were first shown to be immunostimulating in the 1980's. Chitin and chitosan were shown to activate macrophages and natural killer (NK) cells to express a number of pro-inflammatory cytokines such as IL-1, CSF, and IFN-γ; these effects led to enhanced cell-mediated cytotoxicity in mice, in addition to enhancement of antibody production and delayed-type hypersensitivity in guinea pigs (Nishimura *et al.,* 1984, 1985; Iida *et al.*, 1987). In 1986, Suzuki *et al.*, through their analysis of splenic cell changes in cancerous mice, showed that the antitumor mechanism of COS is to enhance acquired immunity by accelerating T-cell differentiation to increase cytotoxicity and maintain T-cell activity.

71. Patel & Goyal (2017) consider that desriptions of chitin having "exceptionally low" immunogenicity (e.g. Zhang *et al.*, 2011) are "misleading". Indeed, there appears to be a more complex picture regarding the immunological properties of chitin. Lee *et al.* (2008) speculated that "when chitin containing pathogens enter a host, the innate anti-pathogen response contains oxidants and chitinases that induce chitin fragmentation. The resulting intermediate sized fragments, in turn, serve as an alarm signal to induce and amplify local inflammation by activating pattern recognition receptors and pathways like NF-κB. This would continue until the invader has been successfully dealt with and smaller chitin fragments are generated. These small fragments would induce molecules like IL-10 which feedback to control the local inflammatory response".

72. Mammalian innate immune responses to chitin seem to depend on the size of the chitin fragments used to stimulate immune cells (Da Silva *et al.,* 2009). Very large (>100 μm) chitin fragments seem to be immunologically inert, while intermediate (40–70 μm) and small chitin (<40 μm) seem capable of activating macrophages and eliciting IL-17, TNF and IL-23 production via a range of pattern recognition receptors (PRRs) (Da Silva *et al.,* 2008). For example, intravenous administration of small chitin particles (1–10 μm) into the lung activated alveolar macrophages to express cytokines such as IL-12, tumour necrosis factor (TNF)-α, and IL-18 (Shibata *et al.*, 1997).

73. Administration of chitin/chitosan beads (administered directly into the lungs, Reese *et al.*, 2007) and microparticles (injected subcutaneously, Heseini *et al.*, 2016) have caused immune responses in mice.

74. Koller *et al.* (2011) showed that epidermal or epithelial cells can recognise chitins via PRRs, leading to cytokine/chemokine secretion. This may be important in the regulation of epidermal immunity, since chitin is expressed by microorganisms that are involved in some skin allergies.

75. The effect of chitosan as a novel adjuvant to an inactivated influenza vaccine was studied (Chang *et al.*, 2004). Here, BALB/c mice were abdominally inoculated with vaccine and chitosan together twice every three weeks. Blood serum was prepared and tested for levels of antibodies IgG, IgG1, and IgG2a as well as IgA antibody in nasal secretions. One week after the immunisation regimen, the mice were challenged with the deadly flu virus A/PR/8/34(H1N1) and the weights of the mice and levels of antibody protection were measured. The results indicated that using chitosan as an adjuvant increased the antibody content in serum remarkably

and increased the antiviral defence in the mice, enhancing the immune reaction to the vaccine.

76. Huang *et al.* (2006) studied the anticancer activities of differently charged COS derivatives using three cancer-cell lines: HeLa, Hep3B, and SW480. Neutral red and MTT cell-viability studies revealed that highly charged COS derivatives could significantly reduce cancer-cell viability, regardless of their positive or negative charge. Furthermore, fluorescence microscopic observations and DNA fragmentation studies confirmed that the anticancer effect of these highly charged COS derivatives were due to necrosis. However, the exact molecular mechanism for the anticancer activity of strongly charged COS compared to their poorly charged counterparts was not clear to the authors.

77. *Lactococcus lactis* and *Lactobacillus plantarum* have chitin-binding and/or chitinolytic proteins (Sánchez *et al.*, 2011). These bacteria are integral part of gut normal flora, fermented foods, and probiotic-fortified foods (Kim *et al.*, 2013; Todorov *et al.*, 2012). However, their inflammatory role in the gut has not been observed, indicating that if chitins accidently reach the gut, they are converted to some other, non-immunogenic form, and thus immune activation in gut does not occur (Patel & Goyal, 2017). Furthermore, Patel & Goyal (2017) stated that "excess chitin exposure is likely to be increasing chitinolytic bacteria in human microbiome".

78. The ability of chitin to activate a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes) has recently been reviewed by Komi *et al.* (2019). Given these immunostimulating effects, Komi *et al.* concluded that:

- wide distribution of chitin makes its exposure inevitable; however, the avoidance of chitin exposure needs to be investigated;
- commercial shellfish chitin has been used in most chitin immunology studies, and our knowledge remains incomplete regarding other sources of chitin such as fungal chitin in similar studies; and,
- lacking novel methods for chitin purification may explain the conflicting data in the literature of immune responses to chitin.

79. Bae *et al.* (2013) investigated the role of chitin and chitosan in inhibition of food allergic responses to peanuts. They treated C3H/HeJ mice with α-chitin, β-chitin, and β-chitosan for 6 weeks starting 1 week before peanut sensitisation. They evaluated the allergic symptoms 30-40 minutes after the oral ground whole peanut challenge, and reported the capability of chitin and chitosan to suppress the anaphylaxis symptoms from peanut-induced hypersensitivities. Moreover, peanut-specific IgE levels were reduced in mice treated with α-chitin and β-chitosan.

Case reports of reactions to chitosan

80. Kato *et al.* (2005) reported a case of immediate-type allergy for chitosan-containing health food. The patient was a 47-year-old female who developed systemic urticaria and difficulty in breathing after oral ingestion of chitosan. Since skin tests (prick test and scratch patch test) were positive, the test was done using another commercial chitosan, and was positive. The patient was diagnosed as

having chitosan-induced immediately-type allergy, and was instructed to avoid ingestion of chitosan. The patient developed no symptoms thereafter. The study authors concluded that chitosan may have functioned as a food allergen because of its molecular weight and general properties. The Committee agreed that the limited information provided in this case report did not suggest any additional concerns. It was considered that this reported case of immediate-type allergy is most likely due to residuals from the shellfish source from which the chitosan supplement was derived.

81. Two case reports were identified relating to hypersensitivity to some healthcare products containing chitosan (Cleenewerck *et al.,* 1994; Pereira *et al.*, 1998). The Committee agreed that the type of hypersensitivity described in these two cases very rarely, if ever, occurs in the context of food ingestion.

Summary and conclusions

82. The risk of allergenicity from chitin- or chitosan-based BBFCMs on the basis of the potential presence of allergenic proteins appears to be low. However, to confirm this, more information is needed, in particular additional data characterising the protein content in chitosan and the final BBFCMs (against chemical and enzymatic methods of deproteination) would be useful, together with data on migration from, and consumption of, BBFCMs. Information on the total amount of residual protein (expressed as mg/g BBFCM) would be helpful for estimating health risks.

83. The available clinical ingestion data indicate that the immunological properties of chitin and chitosan are of low concern in the context of BBCFMs. Chitin is well tolerated in supplements at higher exposures than would be expected from the use of BBFCMs. However, some adverse effects were associated with high intakes of the raw materials in clinical studies, which were typically mild symptoms of gastrointestinal tract distress such as diarrhoea, bloating, or vomiting. It was agreed that these adverse effects were not of concern for BBFCMs as the processing is likely to produce a more inert final material. Furthermore, the phagocytosis of small fragments of chitin or chitosan appeared to be the same as that of similar-sized particles in general.

COT draft statement
September 2021

References

Abdelmalek B.E., Sila A., Haddar A., *et al.* (2017) β-Chitin and chitosan from Squid gladius: biological activities of chitosan and its application as clarifying agent for apple juice. *Int J Biol Macromol* **104:** 953-962

Abdulkarim A., Isa M.T., Abdulsalam S., *et al.* (2013) Extraction and characterisation of chitin and chitosan from Mussel Shell. *Civil and Environmental Research* **3:** 108-114

Arslan B. & Soyer A. (2018) Effects of chitosan as a surface fungus inhibitor on microbiological, physicochemical, oxidative and sensory characteristics of dry fermented sausages. *Meat Sci.* **145:** 107-113

Aytekin O. & Elibol M. (2010) Cocultivation of *Lactococcus lactis* and *Teredinobacter turnirae* for biological chitin extraction from prawn waste. *Bioprocess Biosyst. Eng.* **33:** 393-399

Bae M.J., Shin H.S., Kim E.K., *et al.* (2013) Oral administration of chitin and chitosan prevents peanut-induced anaphylaxis in a murine food allergy model. *Int J Biol Macromol* **61:** 164-168

Bajaj M., Winter J., & Gallert C. (2011) Effect of deproteination and deacetylation conditions on viscosity of chitin and chitosan extracted from *Crangon crangon* shrimp waste. *Biochemical Engineering Journal* **56:** 51- 62

Barennes H., Phimmasane M., & Rajaonarivo C. (2015) Insect consumption to address undernutrition, a national survey on the prevalence of insect consumption among adults and vendors in Laos. *PLoS ONE* **10(8):** e0136458.

Benhabiles M.S., Salah R., Lounici H., *et al.* (2012) Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food Hydrocoll* **29:** 48-56

Berezina N. & Hubert A. (2020) Chapter 19: Marketing and Regulations of Chitin and Chitosan from Insects. In: Chitin and Chitosan: Properties and Applications, First Edition. Van den Broek L.A.M. & Boeriu C.G. (Eds). John Wiley & Sons Ltd.

Bradley E.L. (2010). FSA Project A03070: Biobased materials used in food contact applications: an assessment of the migration potential. Available at: https://www.food.gov.uk/sites/default/files/media/document/a03070.pdf

Bueter C.L., Lee C.K., Rathinam V.A., *et al.* (2011) Chitosan but not chitin activates the inflammasome by a mechanism dependent upon phagocytosis. *J Biol Chem* **286:** 35447-35455

Boot R.G., Blommaart E.F., Swart E., *et al.* (2001). Identification of a novel acid mammalian chitinase distinct from chitotriosidase. *Journal of Biological Chemistry* **276:** 6770-6778

Boot R.G., Bussink A.P., Verhoek M., *et al.* (2005). Marked differences in tissue-specific expression of chitinases in mouse and man. *Journal of Histochemistry and Cytochemistry* **53:** 1283-1292

Campos C.A., Gerschenson L.N., & Flores S.K. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. *Food Bioprocess Technol.* **4:** 849-875

Castillo R., Carrilo T., Blanco C. *et al.* (1994) Shellfish hypersensitivity: clinical and immunological characteristics. *Allergol. Immunopathol.* **22:** 83-87

Chae S.Y., Jang M.K., & Nah J.W. (2005) Influence of molecular weight on oral absorption of water soluble chitosans. *J Control Release.* **102(2):** 383-394

Chang H.Y., Chen J.J., Fang F., *et al.* (2004). Enhancement of antibody response by chitosan, a novel adjuvant of inactivated influenza vaccine. *Chinese Journal of Biologicals* **17(6):** 21-24

Changrkrachang S. (1996) Specification of chitin and chitosan in the world market. Proceedings of workshop on the quality criteria of chitin and chitosan. Bangkok, Thailand.

Chien P.J., Sheu F., & Yang F.H. (2007) Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering* **78:** 225-229

Cleenewerck M.B., Martin P., & Laurent D. (1994). Allergic contact dermatitis due to a moisturizing body cream with chitin. *Contact Dermatitis* **31(3):** 196-197

Da Silva C.A., Hartl D., Liu W., *et al.*, (2008): TLR-2 and IL-17A in chitin-induced macrophage activation and acute inflammation. *J Immunol* **181:** 4279-4286

Da Silva C.A., Chalouni C., Williams A., *et al.* (2009) Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. *J Immunol* **182:** 3573-3582

Daul C.B., Slattery M., Reese G., *et al.* (1994) Identification of the major brown shrimp (Penaeus aztecus) allergen as the muscle protein Tm. *Int. Arch. Allergy Immunol.* **105:** 49-55

De Holanda H.D., & Netto F.M. (2006) Recovery of components from shrimp (Xiphopenaeus kroyeri) processing waste by enzymatic hydrolysis. *J Food Sci.,* **71:** 298-303

Deters A., Petereit F., Schmidgall J., *et al.*, (2008) N-acetyl-d-glucosamineoligosaccharides induce mucin secretion from colonic tissue and inducedifferentiation of human keratinocytes, *J. Pharm. Pharmacol*. **60:** 197-204

Du W.L., Niu S.S., Xu Y.L., *et al.* (2009) Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydr Polym*. **75(3):** 385-389

Duan S., Li L., Zhuang Z., *et al.* (2012) Improved production of chitin from shrimp waste by fermentation with epiphytic lactic acid bacteria. *Carbohydr. Polym*. **89:** 1283-1288

EC (1990) European Commission Directive 90/128/EEC relating to plastics materials and articles intended to come into contact with foodstuffs. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31990L0128&from=en

EC (2011) Regulation (EC) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0010&from=EN

EC (2018) Top 20 innovative bio-based products: Task 3 of "Study on Support to R&I Policy in the Area of Bio-based Products and Services". p. 217-218 Available at: https://op.europa.eu/en/publication-detail/-/publication/15135e98-81c2-11e9-9f05-01aa75ed71a1

EFSA (2010) Scientific Opinion on the safety of 'Chitin-glucan' as a Novel Food ingredient. EFSA Journal 8(7): 1687 Available at: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2010.1687

EFSA (2011) Scientific Opinion on the substantiation of health claims related to chitosan and reduction in body weight (ID 679, 1499), maintenance of normal blood LDL-cholesterol concentrations (ID 4663), reduction of intestinal transit time (ID 4664) and reduction of inflammation (ID 1985) pursuant to Article 13(1) of Regulation (EC) No 1924/20061 *EFSA Journal* **9(6):** 2214
Available at:
https://www.efsa.europa.eu/en/efsajournal/pub/2214

Fabec B., Hellstrom T., Henrysdotter G., *et al.* (2000). Active and intelligent food Packaging. A Nordic report on the legislative aspects. Nordic co-operation, p.29 Available at:
https://books.google.co.uk/books?hl=en&lr=&id=vae6C6FhlkwC&oi=fnd&pg=PA9&ots=w49i3ihn3S&sig=u40Qwk6if-sk1cHNmiTqieMJBgo&redir_esc=y#v=onepage&q&f=false

Faber M.A., Pascal M., El Kharbouchi O., *et al.* (2017) Shellfish allergens: tropomyosin and beyond. *Allergy* **72:** 842-848

Fajardo P., Martins J.T., Fuciños C., *et al.* (2010) Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *J. Food Eng.* **101:** 349-356

Ferreira A.R., Alves V.D., & Coelhoso I.M. (2016) Polysaccharide-based membranes in food packaging applications. *Membranes (Basel)* **6:2**

Funkhouser J.D. & Aronson N.N. (2007) Chitinase family GH18: Evolutionary insights from the genomic history of a diverse protein family. *BMC Evol Biol.* **7(1):** 96

Gades M.D. & Stern J.S. (2003) Chitosan supplementation and fecal fat excretion in men. *Obes Res.* **11(5):** 683-688

Gades M.D. & Stern J.S. (2005) Chitosan supplementation and fat absorption in men and women. *J Am Diet Assoc.* **105(1):** 72-77

Gadgey K.K. & Bahekar A. (2017) Studies On Extraction Methods of Chitin From CRAB Shell and Investigation of Its Mechanical Properties. *International Journal of Mechanical Engineering and Technology* **8(2):** 220-231

Guo Y., Chen X., Yang F., *et al.* (2019). Preparation and characterization of chitosan-based ternary blend edible films with efficient antimicrobial activities for food packaging applications. *Journal of Food Science* **84(6):** 1411-1419

Hahn T., Tafi E., Paul A., *et al.* (2020) Current state of chitin purification and chitosan production from insects. *Journal of Chemical Technology and Biotechnology* **95:** 2775-2795

Han C., Lederer C., McDaniel M., *et al.* (2005) Sensory Evaluation of Fresh Strawberries (Fragaria ananassa) Coated with Chitosan-based Edible Coatings. *Journal of Food Science* **70(3):** S172-S178

Han W., Yu Y., Li J., *et al.* (2011). Application and safety assessment for nano-composite materials in food packaging. *Chinese Science Bulletin* **56(12):** 1216-1225

Healy M.G., Romo C.R., & Bustos R. (1994) Bioconversion of marine crustacean shell waste. *Resour. Conserv. Recycl.* **11:** 139-147

Hu D., Wang H., & Wang L. (2016) Physical properties and antibacterial activity of quaternized chitosan/carboxymethyl cellulose blend films. *LWT-Food. Sci. Technol.* **65:** 398-405

Huang B., Xiao D., Tan B., *et al.*, (2016) Chitosanoligosaccharide reduces intestinal inflammation that involvescalcium-Sensing receptor (CaSR) activation in lipopolysaccharide(LPS)-challenged piglets. *J. Agric. Food Chem.* **64:** 245-252

Huang J.Y., Li X., & Zhou W. (2015). Safety assessment of nanocomposite for food packaging application. *Trends in Food Science & Technology* **45(2):** 187-199

Huang R.H., Mendis E., Rajapakse N., *et al.* (2006). Strong electronic charge as an important factor for anticancer activity of chitooligosaccharides (COS). *Life Science* ***78*****(20):** 2399-2408

Huang Q., Qian X., Jiang T., *et al.* (2019) Effect of chitosan and guar gum based composite edible coating on quality of mushroom (Lentinus edodes) during postharvest storage. *Scientia Horticulturae* **253:** 382-389

Hoffman D.R., Day E.D., & Miller J.S. (1981). The major heat stable allergen of shrimp. *Annals of Allergy* **47(1):** 17-22

Iida J., Une T., Ishihara C., *et al.*, (1987) Stimulation of non-specific host resistance against Sendai virus and *Escherichia coli* infections by chitin derivatives in mice. *Vaccine* **5:** 270-274

Ifuku S. & H. Saimoto (2012) Chitin nanofibers: preparations, modifications, and applications. *Nanoscale* **4(11):** 3308-18

Jeevahan J. & Chandrasekaran M. (2019) Nanoedible films for food packaging: a review. *J. Mater. Sci.* **54:** 12290-12318

Jung W.J., Jo G.H., Kuk J.H., *et al.* (2006) Extraction of chitin from red crab shell waste by cofermentation with *Lactobacillus paracasei* subsp. tolerans KCTC-3074 and Serratia marcescens FS-3. *Appl. Microbiol. Biotechnol.* **71:** 234-237

Jung W.J., Jo G.H., Kuk J.H., *et al.* (2007) Production of chitin from red crab shell waste by successive fermentation with *Lactobacillus paracasei* KCTC-3074 and *Serratia marcescens* FS-3. *Carbohydr. Polym.* **68:** 746-750

Kato Y., Yagami A., & Matsunaga K. (2005) A case of anaphylaxis caused by the health food chitosan. *Arerugi* **54:** 1427-1429

Kim D., Beck B.R., Heo S.B., *et al.* (2013) Lactococcuslactis BFE920 activates the innate immune system of olive flounder(Paralichthys olivaceus), resulting in protection against *Streptococcus iniae* infection and enhancing feed efficiency and weight gain in large-scale field studies. *Fish. Shellfish Immunol.* **35:** 1585-1590

Kim K.M., Son J.H., Kim S.K., *et al.* (2006). Properties of chitosan films as a function of pH and solvent type. *Journal of Food Science* **71(3):** E119-E124

Klinkesorn U. (2013) The Role of Chitosan in Emulsion Formation and Stabilization. *Food Reviews International* **29(4):** 371-393

Koller B., Müller-Wiefel A.S., Rupec R., *et al.* (2011) Chitin modulates innate immune responses of keratinocytes. *PLoS One* 6e16594

Konovalova M.V., Markov P.A., Durnev E.A., *et al.* (2017) Preparation and biocompatibility evaluation of pectin and chitosan cryogels for biomedical application. *J Biomed Mater Res.* ***105*****(2):** 547-556

Komi D.E.A., Sharma L., & Cruz C.S.D. (2019) Chitin and Its Effects on Inflammatory and Immune Responses. *Clinic Rev Allerg Immunol* **54:** 213-223

Kubota N. & Eguchi Y. (1997) Facile preparation of water-soluble N-acetylated chitosan and molecular weight dependence of its water-solubility. *Polym J.* **29(2):** 123

Kumar S., Mukherjee A., & Dutta J. (2020) Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives. *Trends in Food Science & Technology* **97:** 196-209

Kurita K. (2006) Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol*. **8:** 203-226

Lee C.G., Da Silva C.A., Lee J.Y., *et al.* (2008) Chitin regulation of immune responses: an old molecule with new roles. *Curr Opin Immunol* **20:** 684-689

Lehrer S.B., Ayuso R., & Reese G. (2003) Seafood Allergy and Allergens: A Review. *Marine Biotechnology* **5(4):** 339-348

Lopata A.L. & Lehrer S.B. (2009) New insights into seafood allergy. *Current Opinion in Allergy and Clinical Immunology* **9(3):** 270-277

Lopata A.L., O'Hehir R.E., & Lehrer S.B. (2010) Shellfish allergy. *Clinical & Experimental Allergy* **40(6):** 850-858

Lowry O.H., Rosebrough N.J., Farr A.l., *et al.* (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275

Ma M., Qin A, Li X., *et al.* (2014) Bioinspired design and chitin whisker reinforced chitosan membrane. *Materials Letters* **120:** 82-85

Madhumathi K., Shalumon K.T., Rani V.V., *et al.* (2009) Wet chemical synthesis of chitosan hydrogel-hydroxyapatite composite membranes for tissue engineering applications. *Int J Biol Macromol.* **45(1):** 12-15

Manni L., Ghorbel-Bellaaj O., Jellouli K., *et al.* (2010) Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from *Bacillus cereus* SV1. *Appl. Biochem. Biotechnol.* **162:** 345-357

Mincea M., Negrulescu A., & Ostafe V. (2012) Preparation, modification, and applications of chitin nanowhiskers: a review. *Rev. Adv. Mater. Sci.* **30:** 225-242

Mohammed M.H., Williams PA and Tverezovskaya O. (2013) Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocoll* **31:** 166-171

Moreira M.R., Roura S.I., & Ponce A. (2011) Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. *Food Science and Technology* **44:** 2335-2341

Morganti P. (2013) Innovation, nanotechnology and industrial sustainability

by the use of natural underutilized byproducts. *J Mol Biochem* **2(3):** 137-141

Müller K., Bugnicourt E., Latorre M., *et al.* (2017) Review on the Processing and Properties of Polymer Nanocomposites and Nanocoatings and Their Applications in the Packaging, Automotive and Solar Energy Fields. *Nanomaterials* **7:** 74

Muñoz G., Valencia C., Valderruten N., *et al.* (2015) Extraction of chitosan from *Aspergillus niger* mycelium and synthesis of hydrogels for controlled release of betahistine. *Reactive & Functional Polymers* **91-92:** 1-10

Muzzarelli R.A. (2010). Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Mar Drugs* **8(2):** 292-312

Nagpal S., Rajappa L., Metcalfe D., *et al.* (1989) Isolation and characterization of heat-stable allergens from shrimp (*Penaeus indicus*). *Journal of Allergy and Clinical Immunology* **83(1):** 26-36

Nguyen M.X.H. (2012) Characterization of allergenic and antimicrobial properties of chitin and chitosan and formulation of chitosan-based edible film for instant food casing. Melbourne, Australia: Royal Melbourne Institute of Technology (RMIT) University, PhD thesis. Available at: https://core.ac.uk/download/pdf/15625643.pdf

Nishimura K., Nishimura S., Nishi N., *et al.*, (1984) Immunological activity of chitin and its derivatives. *Vaccine* **2:** 93-99

Nishimura K., Nishimura S., Nishi N., *et al.* (1985) Adjuvant activity of chitin derivatives in mice and guinea pigs. *Vaccine* **3:** 379

No H.K.& Meyers S.P. (1995) Preparation and characterization of chitin and chitosan—A review. *J. Aquat. Food Product Technol.* **4:** 27-52

NTP (2017) Technical Report on the Toxicity Study of Chitosan (CASRN 9012-76-4) Administered in Feed to Sprague Dawley [Crl:CD(SD)] Rats. Toxicity Report 93, National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services

Nwe N. & Stevens W.F. (2002) Production of fungal chitosan by solid substrate fermentation followed by enzymatic extraction. *Biotechnology Letters* **24(2):** 131-134

Ortiz JC, Ruiz AT, Morales-Ramos JA, *et al.*, (2016) Insect mass production technologies. In: Insects as Sustainable Food Ingredients. Academic Press, pp. 153-201

Ottsy M.H., Virum K.M., & Smidsred O. (1996) Compositional heterogeneity of heterogeneously deacetylated chitosans. *Carbohydr. Polym*. **29:** 17-24

Ouattara B., Simard R.E., Piette G., *et al.* (2000) Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int. J. Food Microbiol*. **62:** 139-148

Panariello L., Coltelli M.B., Buchignani M., *et al.* (2019) Chitosan and nano-structured chitin for biobased anti-microbial treatments onto cellulose based materials. *European Polymer Journal* **113:** 328-339

Park B.K. & Kim M.M. (2010) Applications of chitin and its derivatives in biological medicine. *Int J Mol Sci* **11:** 5152-5164

Patel S. & Goyal A. (2017) Chitin and chitinase: Role in pathogenicity, allergenicity and health. *International Journal of Biological Macromolecules* **97:** 331-338

Percot A., Viton C., & Domard A. (2003) Optimization of Chitin Extraction from Shrimp Shells. *Biomacromolecules* **4:** 12-18

Pereira F., Pereira C., & Lacerda M.H. (1998). Contact dermatitis due to a cream containing chitin and a Carbitol. *Contact Dermatitis* **38(5):** 290-291

Pillai C.K.S., Paul W., & Sharma C.P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science* **34(7):** 641-678

Pirouz A.A., Selamat J., Iqbal S.Z., *et al.* (2020) Efficient and Simultaneous Chitosan-Mediated Removal of 11 Mycotoxins from Palm Kernel Cake. *Toxins* **12** (115)

Priyadarshi R. & Rhim J.W. (2020) Chitosan-based biodegradable functional films for food packaging applications. *Innovative Food Science & Emerging Technologies* **62:** 102346

Qin C., Li H., Liu Y., *et al.* (2006) Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers* **63:** 367-374

Qin Y., Zhang S., Yu J., *et al.* (2016) Effects of chitin nano-whiskers on the antibacterial and physicochemical properties of maize starch films. *Carbohydrate Polymers* **147:** 372-378

Raafat D. & Sahl H.G. (2009) Chitosan and its antimicrobial potential-a critical literature survey. *Microbial Biotechnol.* **2(2):** 186-201

Rahman A.M., Kamath A., Lopata S., *et al.* (2010). Analysis of the allergenic proteins in black tiger prawn (*Penaeus monodon*) and characterization of the major allergen Tm using mass spectrometry. *Rapid Communications in Mass Spectrometry* **24(16):** 2462-2470

Rajoka M.S.R., Mehwish H.M., Wu Y., *et al.* (2020) Chitin/chitosan derivatives and their interactions with microorganisms: a comprehensive review and future perspectives. *Critical Reviews in Biotechnology* **40:**

Reese G., Ayuso R., & Lehrer S.B. (1999) Tm: An Invertebrate Pan-Allergen. *International Archives of Allergy and Immunology* **119(4):** 247-258

Reese T.A., Liang H.E., Tager A.M., *et al.* (2007) Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* **447:** 92-96

Remington B.C., Westerhout J., Meima M.Y., *et al.* (2020) Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology* **139:** 111259

Riaz A., Lei S., Akhtar H.M.S., *et al.* (2018). Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. *International Journal of Biological Macromolecules* **114:** 547-555

Rinaudo M. (2006) Chitin and chitosan: Properties and applications. *Prog Polym Sci*. **31(7):** 603-632

Romano P., Fabritius H., & Raabe D. (2007). The exoskeleton of the lobster *Homarus americanus* as an example of a smart anisotropic biological material. *Acta Biomaterialia* **3(3):** 301-309

Sahraee S., Ghanbarzadeh G., Milani J.M., *et al.* (2017a) Development of Gelatin Bionanocomposite Films Containing Chitin and ZnO Nanoparticles. *Food Bioprocess Technol.* **10:** 1441-1453

Sahraee S., Milani J.M., Ghanbarzadeh B., *et al.* (2017b) Physicochemical and antifungal properties of bio-nanocomposite film based on gelatin-chitin nanoparticles. *International Journal of Biological Macromolecules* **97:** 373-381

Sánchez B., González-Tejedo C., Ruas-Madiedo P., *et al.* (2011) *Lactobacillus plantarum* extracellular chitin-binding protein and its role in the interaction between chitin, Caco-2 cells, and mucin. *Appl. Environ.Microbiol.* **77(3):** 1123-6

Sannan T., Kurita K., & Iwakura Y. (1975). Studies on chitin, 1. Solubility change by alkaline treatment and film casting. *Die Makromolekulare Chemie* **176(4):** 1191-1195

Sethulekshmi C. (2014). Chitin and its benefits. *International Journal of Advanced Research in Biological Sciences* **1(6):** 171-175

Shibata Y., Foster L.A., Metzger W.J., *et al.* (1997) Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D glucosamine, in mice. *Infect Immun* **65:** 1734-1741

Sicherer S.H., Muñoz-Furlong A., & Sampson H.A. (2004). Prevalence of seafood allergy in the United States determined by a random telephone survey. *The journal of allergy and clinical immunology* **114(1):** 159-165

Singla A.K. & Chawla M. (2001) Chitosan: some pharmaceutical and biological aspects – an update. *J Pharm Pharmacol* **53:** 1047-1067

Sinha S., Tripathi P., & Chand S. (2012). A new bifunctional chitosanase enzyme from *Streptomyces* sp. and its application in production of antioxidant chitooligosaccharides. *Applied Biochemistry and Biotechnology* **167:** 1029-1039

Souza V.G.L., Pires J.R.A., Rodrigues C., *et al.* (2020) Chitosan Composites in Packaging Industry-Current Trends and Future Challenges. *Polymers* **12:** 417

Sriupayo J., Supaphol P., Blackwell J., *et al.* (2005) Preparation and characterization of a-chitin whisker-reinforced chitosan nanocomposite films with or without heat treatment. *Carbohydrate Polymers* **62:** 130-136

Subba R.P.V., Rajagopal D., & Ganesh K.A. (1998) B- and T-cell epitopes of tropomyosin, the major shrimp allergen. *Allergy* **53:** 44-47

Suzuki K., Mikami T., Okawa Y., *et al.* (1986). Antitumor effect of hexa-N-acetylchitohexaose and chitohexaose. *Carbohydrate Research* **151:** 403-408

Synowiecki J. & Al-Khateeb N.A.A.Q. (2000) The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangon crangon processing discards. *Food Chem.* **68:** 147-152

Tapola N.S., Lyyra M.L., Kolehmainen R.M. *et al.* (2008) Safety aspects and cholesterol-lowering efficacy of chitosan tablets. *J Am Coll Nutr.* **27(1):** 22-30

Taylor G. & Wang N. (2018) Entomophagy and allergies: a study of the prevalence of entomophagy and related allergies in a population living in North-Eastern Thailand. *Bioscience Horizons* **11(8)**

Tharanathan R.N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology* **14(3):** 71-78

TNO (Netherlands Organisation for Applied Scientific Research) (2009). Repeated-dose (13-week) oral toxicity study in rats with chitin-glucan. Study Report provided to EFSA by Kitozyme.

Toan N.V. (2009) Production of Chitin and Chitosan from Partially Autolyzed Shrimp Shell Materials. *The Open Biomaterials Journal* **1:** 21-24

Todorov S.D., Leblanc J.G., Franco B., *et al.*, (2012) Evaluation of the probioticpotential and effect of encapsulation on survival for *Lactobacillus plantarum* ST16 Pa isolated from papaya. *World J. Microbiol Biotechnol* **28:** 973-984

US FDA (2011) Nutrition Center for Food Safety Applied. GRAS Notice Inventory - Agency Response Letter GRAS Notice No. GRN 000397. Available at: https://wayback.archive-it.org/7993/20171031010838/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm287638.htm (accessed 10/08/2021)

US FDA (2012) Nutrition Center for Food Safety Applied. GRAS Notice Inventory - Agency Response Letter GRAS Notice No. GRN 000412. Available at: https://wayback.archive-it.org/7993/20171031010540/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm313047.htm (accessed 10/08/2021)

Vargas M., Albors A., Chiralt A., *et al.* (2006) Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. *Postharvest Biology and Technology* **41:** 164-171

Van den Broek L.A.M., Knoop R.J.I, Kappen F.H.J., *et al.* (2015) Chitosan films and blends for packaging material. *Carbohydrate Polymers* **116:** 237-242

Vasile C. (2018) Polymeric Nanocomposites and Nanocoatings for Food Packaging: A Review. *Materials* **11:** 1834

Viarsagh M. S., Janmaleki M., Falahatpisheh H.R., *et al.* (2010). Chitosan preparation from persian gulf shrimp shells and investigating the effect of time on the degree of deacetylation. *Journal of Paramedical Sciences* 1(2)

Waibel K.H., Haney B., Moore M., *et al.* (2011) Safety of chitosan bandages in shellfish allergic patients. *Military Medicine* **176:** 1153-6

Wedmore I., McManus J.G., Pusateri A.E., *et al.* (2006) A special report on the chitosan-based hemostatic dressing: Experience in current combat operations. *J Trauma Acute Care Surg.* **60(3):** 655-658

Wei D., Sun W., Qian W., *et al.* (2009) The synthesis of chitosan based silver nanoparticles and their antibacterial activity. *Carbohydr Res.* **344(17):** 2375-2382

Wu C., Sun J., Zheng P., *et al.* (2019) Preparation of an intelligent film based on chitosan/oxidized chitin nanocrystals incorporating black rice bran anthocyanins for seafood spoilage monitoring. *Carbohydrate Polymers* **222:** 115006

Xia W., Liu P., Zhang J., *et al.* (2010). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids* **25:** 170-179

Xu Y., Gallert, C., & Winter J. (2008) Chitin purification from shrimp wastes by microbial deproteination and decalcification. *Environ. Biotechnol.* **79:** 687-697

Yang Y.M., Hu W., Wang X.D., *et al.* (2007) The controlling biodegradation of chitosan fibers by N-acetylation in vitro and in vivo. *J Mater Sci Mater Med.* **18(11):** 2117-2121

Yadav M., Goswami P., Paritosh K., *et al.* (2019) Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresources and Bioprocessing* **6:** 8

Yin M., Lin X., Ren T., *et al.* (2018) Cytocompatible quaternized carboxymethyl chitosan/poly(vinyl alcohol) blend film loaded copper for antibacterial application. *Int J Biol Macromol* **120:** 992-998

Ylitalo R., Lehtinen S., Wuolijoki E., *et al.* (2002) Cholesterol-lowering Properties and Safety of Chitosan. *Drug Res.* **52(1):** 1−7

Younes I., Ghorbel-Bellaaj O., Nasri R., *et al.* (2012) Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinisation. *Process Biochem* **47(12):** 2032-2039

Younes I. & Rinaudo M. (2015) Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications Mar. *Drugs* **13:** 1133-1174

Zachetti V.G.L., Cendoya E., Nichea M.J., *et al.* (2019) Preliminary Study on the Use of Chitosan as an Eco-Friendly Alternative to Control *Fusarium* Growth and Mycotoxin Production on Maize and Wheat. *Pathogens* **8:** 29

Zhang H., Li R., & Liu W. (2011) Effects of Chitin and Its Derivative Chitosan on Postharvest Decay of Fruits: A Review. *Int. J. Mol. Sci.* **12:** 917-934

Zhang H., Jung J., & Zhao Y. (2016) Preparation, characterization and evaluation of antibacterial activity of catechins and catechins-Zn complex loaded beta-chitosan nanoparticles of different particle sizes. *Carbohydr Polym.* **137:** 82-91

Zeng J.B., He Y.S., Li S.L., *et al.* (2012) Chitin whiskers: an overview. *Biomacromolecules* **13(1):** 1-11

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Potential allergenicity of chitosan in food contact materials - Additional information and first draft statement

Annex 2: Methods for recovery of chitin from marine resources, and degree of deproteination (DP).

Method for deproteination	Conditions of deproteination	DP (wt %)	References
chemical	shrimp shells; "partial autolysis", then 0.62M NaOH (1:5 w/v) for 20 hours at ambient temperature; 5 samples	99.16 ± 0.12 – 99.45 ± 0.06	Toan (2009)
chemical	Shrimp shells; 1M NaOH for 24 h at 70°C	>99	Percot *et al.* (2003)
chemical	shrimp shells; 2M NaOH for 2-5 hours at 30-65°C; 4 samples, at varying shell:NaOH ratios	95.34 ± 0.38 – 96.83 ± 0.17	Bajaj *et al.* (2011)
chemical	shrimp waste; 1.25M NaOH at ratio of 1:20 (*w/v*) for 4 hours at 80°C	93.8 ± 1.38	Manni *et al.* (2010)
enzymatic	A21 protease enzyme/substrate 7, 75 U/mg (60 °C, 6 h)	88	Younes *et al.* (2012)
enzymatic	Alcalase (50 °C, 3 h)	80	Abdelmalek *et al.* (2017)
enzymatic	Sil-Al 4 × 4 TM inoculant, glucose, 30 °C, 7 days	91	Manni *et al.* (2010)
enzymatic	*S. marcescens, L. paracasei,* glucose, 30 °C, 7 days	68.9	Jung *et al.* (2007)
enzymatic	*L. acidophilus* SW01, glucose, 37 °C, 168 h	96.5	Duan *et al.* (2012)
enzymatic	Stabisil inoculant, lactose, 25 °C	40	Healy *et al.* (1994)
enzymatic	*L. lactis, T. turnirae,* glucose, 7 days	95.5	Aytekin & Elibol (2010)
enzymatic	*L. paracasei, S. marcescens*, glucose, 30 °C, 7 days	52.6	Jung *et al.* (2006)

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Potential allergenicity of chitosan in food contact materials - Additional information and first draft statement

Annex 3: Estimation of shellfish protein in chitin and chitosan based BBFCMs

1. The estimates use 1) the highest reported measured amount of shrimp protein in crustacean-derived chitin and chitosan, and 2) the stated amounts of chitin and/or chitosan in the compositions of some BBFCMs. The estimates below are thus likely to overestimate actual exposure, as they assume that the concentration of shellfish protein in chitin or chitosan is unaffected by the processing which generates the final BBFCM. The amount of BBFCM that would contain shellfish protein equivalent to the ED01 is also estimated for each BBFCM (assuming 100 % migration into food).

2. The % (wt) of shellfish protein in some non-edible BBFCMs in Table 1 is estimated using the following information:

- The protein content of commercial chitin being ≤ 3-4 % (w/w), a percentage range also noted by Changrkrachang (1996) (as cited in Nguyen, 2012). Thus, a percentage of 4 % protein content in commercial chitin is used.
- The protein concentration in the extraction from technical chitosan ranged from 0.05 to 1.0 % (w/w) (Nguyen, 2012). Thus, a percentage of 1 % protein content in chitosan is used.

Table 1: Estimated concentrations of shellfish protein in some **non-edible** BBFCMs, based on chitin nano-whisker and/or chitosan content.

Material type	% Concentration of chitin and/or chitosan in BBFCM	Matrix material/ solvent	Literature reference	Estimated % (wt) shellfish protein in BBFCM*	Estimated amount of BBFCM that would contain shellfish protein equivalent to the ED01 (nearest gram)**
Chitosan film	5 % (w/v) chitosan	carboxy-methyl cellulose	Hu *et al.* (2016)	0.05	52 grams
Film with chitosan and chitin nano-whiskers	2 % (w/v) chitosan and 1 % (wt) chitin nano-whiskers	chitosan	Ma *et al.* (2014)	0.06	44 grams
Film with chitin nano-whiskers	Up to 5 % (wt) chitin nano-whiskers	maize starch	Qin *et al.* (2016)	0.2	13 grams
Film with chitin nano-whiskers	Up to 10 % chitin nano-whiskers	gelatin	Sahraee *et al.* (2017b)	0.4	7 grams

Film with chitosan and chitin nano-whiskers	2 % (w/v) chitosan and up to 29.6 % (wt) chitin nano-whiskers	chitosan	Sriupayo *et al.* (2005)	1.2	2 grams

*Assumes %(wt) of shellfish protein in chitin nano-whiskers and chitosan is 4 % and 1 %, respectively. E.g. if 5 % BBFCM is chitin nano-whiskers, and 4 % of chitin nano-whiskers is protein, then % wt of BBFCM composed of shellfish protein is 5 % x 4 % = 0.2 %.
** Uses the ED01 of 26.2 mg for shrimp protein, where <1 % of the allergic population may be expected to react (Remington *et al.*, 2020). E.g. if 0.2 % (wt) of BBFCM is shrimp protein, then 26.2 mg ÷ 0.2 % = 13.1 g of BBFCM which contains shellfish protein equivalent to the ED01.

3. Table 2 shows some "edible" BBFCMs and their percent chitosan compositions. The emerging chitosan-based films/coatings for fruits, vegetables, fish, and meat products have been reviewed (Wang *et al.*, 2018; Kumar *et al.*, 2020), and the % chitosan concentration across the different films/coatings (which as described as "edible") are generally ≤ 2.0 % (w/v). The % (wt) of shellfish protein in the overall BBFCM is estimated by assuming a 1 % protein content in chitosan, and assuming that the concentration of shellfish protein in chitosan is unaffected by the processing which generates the final BBFCM. The estimated consumption of edible film required reach the ED01 in terms of shellfish protein content is estimated for each BBFCM. No films with chitin or chitin nano-whiskers that were identified in the literature were described as "edible".

Table 2: Estimated concentrations of shellfish protein in some "**edible"** BBFCMs, based on chitosan content.

Material type	**% Concentration of chitosan in BBFCM**	**Matrix material/ solvent**	**Literature reference**	**Estimated % (wt) shellfish protein in BBFCM***	**Estimated consumption of BBFCM to reach ED01 (nearest gram)****
Edible chitosan film	1 % (w/v) chitosan	gelatin	Guo *et al.* (2019)	0.01	262 grams
Edible chitosan coating	1 % (w/v) chitosan	glycerol	Han *et al.* (2005)	0.01	262 grams
Edible chitosan coating	1 % (w/v) chitosan	acetic acid	Vargas *et al.* (2006)	0.01	262 grams
Edible chitosan coating	1 % (w/v) chitosan	acetic acid	Huang *et al.* (2019)	0.01	262 grams
Edible chitosan film	2 % (w/v) chitosan	glycerol	Riaz *et al.* (2018)	0.02	131 grams
Edible chitosan coating	Up to 2 % chitosan	acetic acid	Chien *et al.* (2007)	0.02	131 grams
Edible chitosan coating	2 % (w/v) chitosan	acetic acid	Moreira *et al.* (2011)	0.02	131 grams

*Assumes %(wt) of shellfish protein in chitosan is 1 %. E.g. if 1 % BBFCM is chitosan, and 1 % of chitosan is shellfish protein, then % wt of BBFCM composed of shellfish protein is 1 % x 1 % = 0.01 %.
**Uses the ED01 of 26.2 mg for shrimp protein, where <1 % of the allergic population may be expected to react (Remington *et al.*, 2020). E.g. if 0.01 % (wt) of BBFCM is shrimp protein, then 26.2 mg ÷ 0.01 % = 262 g of BBFCM consumed to reach ED01.

Glossary

BACN - black rice bran anthocyanins
BBFCM - bio-based food contact material
Cmax - maximum concentration
COS - chitooligosaccharide
CSF - colony stimulating factor
DD - degree of deacetylation
DP - degree of deproteination
ED - eliciting dose
ELISA - enzyme-linked immunosorbent assay
FCM - food contact material
FT-IR - Fourier-transform infrared
GMP - good manufacturing practice
GNCF - gelatin-based bionanocomposite film
GRAS - generally recognised as safe
ICPMS - inductively coupled plasma mass spectrometry
Ig - immunoglobulin
IL - interleukin
IFN-γ - interferon-γ
kDA - kilodaltons
LC-MS - liquid chromatography-mass spectrometry
LOD - limit of detection
NDNS - national diet and nutrition survey
NK cell – natural killer cell
NMR - nuclear magnetic resonance
OML - overall migration limit
PLA - poly(lactic) acid
ppb - parts per billion
PRR - pattern recognition receptor
SDS-PAGE - sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SML - specific migration limit
SPT - skin prick test
Tm - tropomyosin
TNF - tumour necrosis factor
Wt - weight