FOOD
RESEARCH

A review on production, application, and toxicological analyses of nanocrystalline cellulose as a novel fat replacer food additive

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Abstract

Nanocrystalline cellulose has gained much attention from the researcher. It provides many applications such as polymer additive, packaging, and tablet filler. This review established the recent isolation method of nanocrystalline cellulose from agricultural by-products, aiming to reduce waste that will affect the environment. The current nanocrystalline cellulose application as a food additive in many food products and its toxicological analyses was discussed. This work can be used as a guideline by researchers and food manufacturers to isolate nanocrystalline cellulose from agricultural waste and its use as a fat replacer in food products.

1. Introduction

Cellulose is the building block of the cell wall in plants, which protects and provides tensile strength to the plant cell wall. Researchers are becoming more interested in cellulose due to its abundance in nature. Moreover, cellulose is a renewable, biodegradable, and nontoxic polymer. This natural polymer has high mechanical strength and a high strength-to-weight ratio while allowing flexibility to counter significant dimensional change due to swelling and shrinking (Dufresne, 2013). Cellulose is an excipient in various industries, such as those involving veterinary foods, wood and paper, fibres and clothes, and cosmetic and pharmaceutical industries (Shokri and Adibkia, 2013). The benefits of cellulose can be further extended when cellulose chains are bundled together, generating highly ordered regions that can be subsequently isolated to produce cellulose nanomaterials or nano cellulose. Nanocellulose can be categorized into three major classes: nanocrystalline cellulose (NCC), nanofibrillated cellulose (NFC), and bacterial nanocrystalline cellulose (BNC) (Phanthong *et al*., 2018). This nanocellulose has similarities in chemical composition but has different morphology, particle size, crystallinity, and extraction method (Lavoine *et al*., 2012).

Awareness of environmental conservation has increased research activity in the search for new materials (Aridi *et al*., 2021). The agricultural sector produces an enormous volume of by-products, and they are generally burnt in the fields, causing air pollution and its accumulation in the soil. It is the most abundant natural fibre, yet a promising and sustainable material for food packaging. This by-product is defined as residues coming from the growing and processing of raw agricultural products, such as fruits, vegetables, meat, dairy products, and crops. It is estimated that about 998 million tons of agricultural by-products are produced yearly (Obi *et al*., 2016).

Recently, many pieces of research have been made by isolating cellulose from agricultural by-products,

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water plants, grasses, and other plant substances, annual plants, microbes, and animals (Ngo, 2019). Table 1 presented the previous research on the use of agricultural by-products for the extraction of nanocellulose. These include potato peel, maize stalk, sago seed shall and many more. Besides that, the percentage of the chemical composition of the new source for cellulose isolation are presented in Table 2. The chemical composition of cellulose, hemicellulose and lignin is depending on the sources and the isolation method used, as in Table 1, *Calotropis Procera fibre (CPF)* has the higher percentage of cellulose which is 64.1%, while banana ranches show the lowest percentage of cellulose, which is only 7.5%.

Various form of cellulose has been approved by the Food and Drug Administration (FDA) for many consumer products in the food and medical industries (Seddiqi *et al.*, 2021). Cellulose has recently become a trendy choice as an additive due to the rising awareness of fibre intake in the food industry. Cellulose gel can act similarly to an emulsion, suspending ingredients within a solution and preventing water from separating. It is often used in sauces (Alam *et al*., 2009) and ice cream (Sebayang and Sembiring, 2017) to act as a thickening and emulsifying agent. Now, engineered materials such as nanocellulose are often added to foods to improve its quality acting as a Pickering emulsion stabilizer (Hedjazi and Razavi, 2017), food packaging (Anžlovar and Kunaver, 2018), as a thickener in printing ink (May *et al*., 2021) and many more other applications.

Nanocrystalline cellulose (NCC) can be extracted from almost any cellulosic material. The research on nanosized bio-based cellulosic materials has been studied in recent years because of its unique combination of their properties which include outstanding mechanical properties, surface chemistry, nontoxicity, biocompatibility, and most importantly, they are abundance from renewable and suitable resources (Ngo *et al*., 2019).

NCC can be used in food areas as a food additive, food coating, and food-contact packaging materials (Ede *et al.,* 2020). This review paper aims to discuss the method and examples of agricultural by-products that have been used for the production of nanomaterials. It will also highlight NCC's recent application as a fat replacer in various types of food products. To date, there is a limited review that is focused on the application of NCC as a fat replacer in the food industry. Thus, this review article focused on how NCC can be used as a fat replacer in ice cream, meat products and mayonnaise. The toxicology analysis of NCC is also discussed.

2. Production of nanocrystalline cellulose (NCC)

Nanocellulose can be categorized into nanocrystalline cellulose (NCC) and nanofibrillated cellulose (NFC). The percentage of cellulose vary depending on the source of the materials. The process of isolating nanocellulose from cellulose-based materials are summarized in Figure 1.

Due to the unique physical, chemical, and thermal properties and the possibility for future nanocrystalline cellulose (NCC) applications, studies on NCC extraction from agricultural wastes have increased. Figure 1 shows the schematic diagram of nanocrystalline cellulose extraction from agricultural wastes, consisting of two steps. These two steps include removing non-cellulosic materials, such as lignin, hemicellulose, and other compounds, via a pre-treatment process, followed by its extraction from cellulose fibrils via chemical or enzymatic extraction methods.

Table 1. Previous research on the use of agricultural by-products for the extraction of nanocellulose

Source	Methods	Type of nanocellulose	Length (nm)	Width (nm)	Reference
Potato peel	Alkali treatment and acid hydrolysis	Nanocrystal cellulose	410	10	Chen *et al.* (2012)
Maize stalk	Alkali treatment, acid hydrolysis, and refrigerated centrifuge	Cellulose Nano whiskers	150–450	3–7	Motaung and Mtibe (2015)
Sago seed shell	Acid hydrolysis	Nanocrystal cellulose	N/A	10–15	Naduparambath
Agave tequilana Weber	Alkali treatment and acid hydrolysis	Nanocrystal cellulose	Rod: 590	Rod: 10 Sphere: 20 Porous: 80	Pech-Cohuo *et al*. (2018)
Rice husk	Alkali treatment, bleaching and ball milling	Nanocrystal cellulose	N/A	15–20	Fathi *et al.*
Calotropis procera	Alkali pre-treatment, delignification, acid hydrolysis	Nanocrystal cellulose	250	12	Song *et al.* (2019)
Walnut shell	Alkali treatment followed by acid hydrolysis and TEMPO oxidation	Nanocrystal cellulose	55–82	49–81	Zheng *et al.* (2019)

Table 2. Percentage chemical composition in different plants

| Plant/source	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Banana ranchis	7.5	74.9	7.9	Moreno *et al.* (2018)
Calotropis procera fibre (CPF)	64.1	19.5	9.7	Song *et al.* 2019)
Kenaf	63.4	17.6	12.7	Kargarzadeh and Ahmad (2012)
Oil palm mesocarp fibre (OPMF)	28.2	32.7	32.4	Megashah *et al.* (2018)
Oil palm frond (OPF)	45.0	32.0	16.9	Nordin *et al.* (2017)
Sugarcane bagasse	43.6	27.7	27.7	Kumar *et al.* (2014)
Sugar palm fibre	43.9	7.24	33.2	Ilyas *et al.* (2017)
Walnut shell	27.4	31.3	36.3	Zheng *et al.* (2019)
Wheat straw	43.2	34.1	22.0	Alemdar and Sain (2008)
Tea leaf	16.2	68.2	18.8	Abdul Rahman *et al.* (2017)
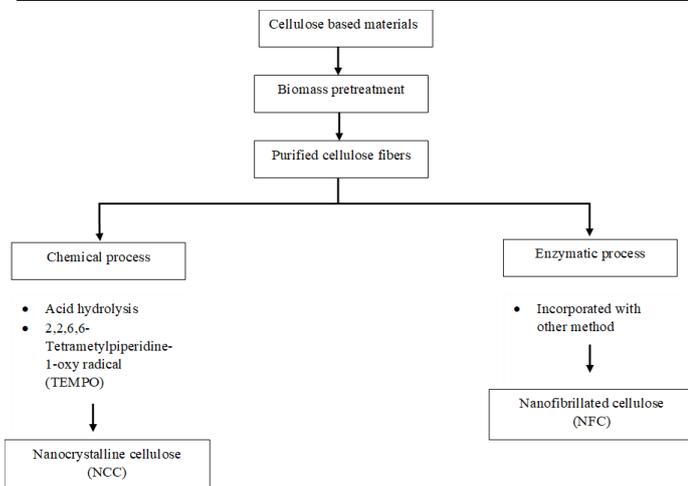
Figure 1. Schematic of the nanocellulose production process

2.1 Biomass pre-treatment

The typical NCC production process using chemical processes is acid hydrolysis, quenching, centrifugation, dialysis, and homogenization to generate purified NCC suspensions followed by drying (Ngo, 2019). However, before beginning NCC isolation, the cellulose-based materials, such as agricultural by-products, are collected and under a pre-treatment process. The use of agricultural by-products as the starting material for NCC production is good for the environment. However, since it consists of cellulose and non-cellulosic components, a pre-treatment step is essential to remove non-cellulosic materials while remaining cellulosic materials extract NCC further. There are two methods for biomass pre-treatment, which are acid-chlorite treatment and alkaline treatment.

The acid-chlorite treatment is also known as the delignification or bleaching process. This process involves removing lignin and other components by stirring the lignocellulosic biomass with a mixture containing distilled water, sodium chlorite, and acetic acid. The mixture is heated at 70°C to 80°C between 4 to 12 hrs (Mandal and Chakrabarty, 2011; Souza *et al.*, 2017; Phanthong *et al.,* 2018). Subsequently, the holocellulose mixture is washed with distilled water until pH 7 is reached. The solid products obtained (i.e., holocellulose) from the process are collected and dried in an oven at 50°C, producing white colour fibre that indicates the successful removal of lignin and other impurities (Abdul Rahman *et al.*, 2017).

The other pre-treatment step is the alkaline treatment. An alkaline solution is used to remove the amorphous region of hemicellulose and the remaining lignin. Generally, sodium hydroxide is used and stirred with holocellulose for 1 to 5 hrs (Fathi *et al.*, 2018). The obtained solid products are then washed with distilled water until pH 7 is reached, followed by drying in the oven at 50°C. The obtained fibre is in cellulose as the amorphous region, while other non-cellulosic materials would have been removed (Li *et al.*, 2012; Souza *et al.*, 2017; Fathi *et al.,* 2018).

Recently, most researchers have been adopting these pre-treatment processes to remove non-cellulosic components from agricultural waste. For example, the percentage of cellulose in a walnut shell before pre-treatment had increased from 27.4% to 87.9% after its pre-treatment with alkaline (Zheng *et al.*, 2019). Additionally, a higher content (from 58.8% to 87.9%) of cellulose in tea leaf waste fibre was obtained after employing the alkaline treatment (Abdul Rahman *et al.*, 2017). These results strongly show that the treatments were efficient in removing most of the hemicellulose and lignin, which results in high cellulose content.

2.2 Nanocrystalline cellulose extraction

Several techniques have been employed in the extraction of nanocrystalline cellulose (NCC) from cellulosic materials. The different extraction procedures have resulted in different types and properties of the obtained nanocrystalline cellulose (Phanthong *et al.,* 2018). In this section, two main extraction procedures, namely, chemical hydrolysis and enzymatic hydrolysis, are discussed. Chemical methods such as acid hydrolysis and the usage of 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO) are usually used to extract NCC. Details of each extraction method are discussed in section 2.2.1 until section 2.2.2.

2.2.1 Chemical extraction

Acid hydrolysis is an easy and convenient process to isolate NCC from the cellulosic fibre. The acid hydrolysis processes need to go through very harsh reaction conditions, which usually involve concentrated acid. During the acid hydrolysis process, the amorphous cellulose regions are more easily invested by acid than the crystalline regions, leading to the first degradation of amorphous regions. In contrast, the crystalline regions are retained (Xie *et al*., 2018) because crystalline regions are primarily insoluble in acids (Ng *et al.,* 2015). Concentrated acid, such as sulphuric and hydrochloric acids, is usually used to release hydronium ions for the hydrolytic cleavage of glycosidic bonds in the cellulose chain, therefore breaking down the structure of the nanofibrils into crystalline nanocrystals. However, the obtained NCC's quality and properties depend on the reaction temperature and acid concentration (Camacho *et al.,* 2017; Phanthong *et al.,* 2018). A few strong acids can be used to degrade cellulosic fibre, but hydrochloric and sulphuric acids have been extensively used.

Nanoparticle that is produced via chemical extraction occurs as high aspect ratio rod-like nanocrystals or whiskers. The geometrical dimensions are highly dependent on the origin of the cellulose substrate and hydrolysis conditions. This can be observed in Table 1 as a different source of NCC will be produced with different lengths and diameters. They are generally present with a relatively broad distribution in length because of the acid hydrolysis's diffusion-controlled nature. The average length is generally a few hundred nanometres, and the width is a few nanometres. Acid hydrolysis is the most popular method for isolating NCC because it causes the obtained NCC to have a unique surface property of SO_3^- that makes NCC have a good dispersion in water solvent (Yu *et al*., 2013). However, one of the drawbacks of acid hydrolysis is the wastewater generated during the washing process. The washing process is usually performed by adding cold water into the hydrolysed solution, followed by centrifugation until a neutral pH is reached. It can also be done using an alkaline solution, such as sodium hydroxide, for neutralization of the material obtained. Previous work had investigated the time-dependent acid hydrolysis of pineapple peel residue to obtain NCC (Camacho *et al.,* 2017).

It is important to remove non-cellulosic material before proceeding with acid hydrolysis because cellulose fibres exposure to a hydrolysing agent is determinant to the NCC production. Thus, non-cellulosic material such as lignin and hemicellulose, which induce a shield effect to the cellulose must be removed first. Besides that, the geometrical aspect ratio of NCC, which is defined as the ratio of length to diameter (L/D), is very important in defining the reinforcing capability of NCC produced (George and Sabapathi, 2015). The higher the aspect ratio, the higher is the reinforcement capacity when incorporated in composite materials.

It was reported that the fibre-like particles had decreased in size between 20 and 60 mins of reaction time. The particles obtained after 60 mins had a nanofiber-like morphology and became more whisker, with round-like morphology after 75 mins. Several examples of NCC produced via acid hydrolysis can be observed, such as NCC production using *Acacia mangium* as the substrate (Jasmani and Adnan, 2016). NCC produced 64% of sulphuric acid hydrolysis from this previous study, resulting in needle-like NCC with 79% crystallinity. Moreover, the same percentage of sulphuric acid was used to extract NCC from sago seed shells (Naduparambath *et al.,* 2017). However, the percentage of crystallinity obtained was slightly lower, with only 72% compared with the NCC produced from *Acacia mangium.*

Nevertheless, there was also research that had utilized 60% of sulphuric acid for the hydrolysis process. The NCC was isolated from sugar palm fibres using 60% of sulphuric acid (Ilyas *et al*., 2017), in which a high crystallinity of NCC was obtained at 85.9%. This proved that the physical and chemical properties of NCC obtained are highly dependent on the origin of the material used in the extraction process and the condition of the extraction process involved. Different percentages of hydrolysis agent and species of materials used will produce a different percentage of crystallinity.

Moreover, the second method of isolating NCC is by using an oxidation process. According to previous literature, 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation was introduced to reduce the energy and waste produced (Zheng *et al.,* 2019). TEMPO is a stable and commercially available organic-free radical reagent used to oxidize primary alcohol into aldehydes. Furthermore, TEMPO is used as a catalyst, with hypochlorite as the primary oxidant for oxidizing the hydroxyl groups of cellulose into carboxylates. Several studies compare the properties of NCC obtained using chemical hydrolysis and TEMPO. The NCC produced by TEMPO-mediated oxidation has unique properties in a uniform structure, having ultrafine widths between 3–4 nm, high crystallinity, and a high aspect ratio. From previous work, the isolation of NCC from softwood bleached kraft pulp and microcrystalline cellulose using TEMPO exhibited higher recovery ratios and smaller and more homogeneous width (Zhou *et al*., 2018). However, NCC obtained from the walnut shell via TEMPO showed a different finding. The NCC obtained

using TEMPO-mediated oxidation from walnut shells had irregular block structures, whereas NCC obtained by acid hydrolysis had rectangular block structures (Zheng *et al.,* 2019). Other literature on the use of TEMPO oxidation to produce NCC is presented in Table 3.

2.2.2 Enzymatic hydrolysis

Nanocellulose that is obtained by enzymatic hydrolysis is known as cellulose nanofiber (CNF) (Börjesson and Gunnar, 2015). This process does not generate toxic residue other than acid hydrolysis (Ribeiro *et al.*, 2019). The biological treatment is performed in mild conditions; however, a long time is required for this process. It is a costly process to obtain NCC via enzymatic degradation, but it can be improved to obtain a more economically process for large-scale production. Once the cellulose crystal is obtained from the crystalline region, the selectivity of the endoglucanase enzyme is used to explore the amorphous regions, which then yield NCC, while the crystalline region is fragmented into CNF by mechanical treatments such as ultrasonication.

During the enzymatic hydrolysis of a cellulosic material, the reaction rate gradually decreases until the reaction stops. Several factors can affect the reaction rate related to both the enzyme and the substrate itself (Penttilä *et al.*, 2013). Besides that, the tight association of cellulose microfibrils and non-cellulosic materials, such as hemicellulose or lignin, can be the limiting factors that slow down the hydrolysis process.

The isolation of NCC from *Amorpha fruticosa* can be done via an enzyme-assisted pre-treatment, having successfully isolated the nanocrystalline cellulose of a diameter of approximately 10 nm, while the aspect ratio is over 1,000 (Zhuo *et al.,* 2011). A study on the production of NCC from citrus waste using enzymatic hydrolysis had produced NCC with a high aspect ratio (Mariño *et al.*, 2015). It was observed that the obtained NCC exhibited 55% of crystallinity, with an average diameter of 10 nm.

3. Application of nanocrystalline cellulose as additive low-fat food products

Over the past few decades, the Dietary Guidelines for Americans has consistently recommended that consumers decrease their consumption of saturated fatty acids due to the correlation of saturated fatty acid intake with coronary artery disease. This recommendation has not been easy to achieve because saturated fatty acids play an essential role in the quality, shelf life, and acceptability of foods (Vieira *et al.*, 2015). Fats and oils are essential ingredients in foods, as they provide desirable characteristics, which contribute to the tenderness and aeration of the batter while adding flavour to foods. Food texture is affected by fats via crystalline networks' structuring and by the disruption of structure through interference with non-fat networks.

Many researchers have conducted studies that demonstrated important nanocellulose applications in a variety of food formulations, especially as a fat replacer. In Asia, nanocellulose such as nanobacteria cellulose, nanofibrillated cellulose and nanocrystalline cellulose is generally used in baked goods, desserts, snacks, drinks, and beverages as a food ingredient, a popular gelatinous and springy fibre (Aylin Akoğlu *et al.*, 2018). Section 3.1, 3.2, and 3.3 discusses and summarises nanocellulose application as a fat replacer in food products.

3.1 Nanocellulose in ice cream

Ice cream is a complex food colloid containing fat globules, air bubbles, and ice crystals dispersed in an aqueous phase of high viscosity and consisting of proteins, salts, sugars, and polysaccharides. It usually contains about 30% of ice, 50% of air, 5% of fat, and 15% sugar solution in volume. It requires an emulsifier, as the emulsions are thermally unstable systems and thus require energy to increase the surface area between the oil phase and water. The setting up of the ice cream structure comes from the manufacturing process and the various components used in the formulation. Fat appears to contribute mainly to the properties of ice cream during freezing and whipping through the partially coalesced continuous three-dimensional network of homogenized

Table 3. Previous research on isolating nanocrystalline cellulose via TEMPO oxidation process

Material	Characteristics	Reference
Softwood bleached kraft pulp	• High mass recovery ratio • High amount of surface anionic groups • Small and more homogeneous width	Zhou *et al.* (2018)
Walnut shell	• Irregular block structure • High crystallinity index	Zheng *et al.* (2019)
Oil palm empty fruit bunch	• Crystallinity index ranges between 34% and 55% • High aspect ratio • High presence of carboxylate group	Hastuti *et al.* (2019)
Blueberry pruning residue	• Rod-like shape • High colloidal stability • High crystallinity	Pacheco and Reyes (2020)

globules, which helps in air phase stabilization. This is because fat globules surround air bubbles, and increased fat aggregation levels are also correlated to improved melting resistance (Rios *et al.*, 2014). Fats provide flavour, colour, texture, and mouthfeel to the ice cream. Fat is the primary carrier of flavour. Therefore, reducing fat content in the ice cream will result in the loss of texture and sensory properties.

Preliminary results already show that bacterial nanocellulose (BNC) is technically superior to these vegetable celluloses and can outperform plant celluloses in several food industry applications. BNC presents essential features as a novel hydrocolloid, such as stabilizing heterogeneous systems (air-liquid, solid-liquid, and liquid-liquid). It can stabilize aerogels and increase the incorporation of air in a liquid matrix (overrun). These properties are suitable to be used as an additive in ice cream, smoothies, and whipped cream. Besides, BNC is also used as a stabilizer. It can stabilize solid particles in a liquid matrix (e.g., cocoa particles in chocolate milk) and stabilize oil-in-water emulsions in spoonable and pourable dressings without the need to add any other emulsifying agents (Daniela Martins *et al.*, 2017).

Previous studies have investigated nano bacterial cellulose (NBC) usage on textural properties isolated from soy protein as a fat replacer in ice cream (Guo *et al.*, 2018). The nano bacterial cellulose used in this study was produced by *Gluconacetobacter xylinus*. Thermal stability, textural, rheological, and emulsifying properties of Nano-BC/SPI mixtures were improved in ice cream compared with that of pure soy protein isolate (SPI). The ice cream formulation consisted of 45% whole milk, 15% white sugar, 10% yolk, and 30% cream. However, the percentage of cream was varied at 10%, 20%, and 30%, and replaced with the equivalent amount of NBC/SPI mixture. From the data obtained, the addition of NBC showed no significant difference in the thermal behaviours of the ice cream. It was concluded that 20% of NBC/SPI had similar textural properties to the ice cream. Moreover, recent research has investigated the addition of cellulose nanofibrils (NFC) in ice cream formulation (0.15% and 0.3 wt.% of NFC) on the structural elements of the ice cream. Velásquez-Cock *et al.,* (2018) reported that the sensory properties of low-fat ice cream had improved after the addition of NFC, even after the specimen was given a heat shock.

3.2 Nanocellulose in meat products

Developing lean or extra lean products while assuring consumers' necessary palatability is not as simple as just removing fat from the product. The active approach to fat replacement is to add fat mimetic ingredients, such as carbohydrates or proteins, either to replace fat or modify the remaining compounds' interactions. Numerous non-meat ingredients are available today as texture-modifying and water-binding agents. While not always marketed, they act as fat replacers, modifying texture and mouthfeel and binding water to retain juiciness. Successful fat reduction in meat and poultry products requires a good understanding of fat in meat products and how those functions can be replicated with non-fat ingredients. A successful fat replacement product must replicate the texture, mouthfeel, and flavour of the original fat. The critical properties of meat products that are influenced by fat include appearance, mouthfeel, texture/juiciness, flavour, and storage stability. Fat affects the texture, tenderness, and bite of meat products. It also contributes flavour and serves as a carrier for fat-soluble flavours. In meat products, juiciness and moisture release are often affected by changes in the fat level.

Reformulated meat products have been created to help consumers who are continually requiring nutritionally improved meat products, with a lower content of fats, cholesterol, sodium chloride, and nitrites and a higher content of compounds beneficial to human health. Sodium chloride (currently named salt) is widely used in meat products due to a series of technological benefits, such as increasing the water-binding capacity of proteins and improving the texture and shelf life. Recent research on the application of nanocellulose as a fat replacer was done on nanofibrillated cellulose in an emulsified sausage (Wang *et al.,* 2018). They compared 1 wt.% aqueous dispersion of nanofibrillated cellulose (NFC) and its palm oil Pickering emulsion (CPOE) at the ratio of 1:1 (water: oil, v/v) for being fat alternatives by replacing 30% and 50% of the original fat of the emulsified sausage. From the data obtained, the addition of NFC (30%) in the emulsified sausage resulted from a better texture profile, with an increase in hardness, springiness, and chewiness. Besides that, the addition of NFC in emulsified sausages showed higher sensory scores and therefore concluding that NFC has the potential to be used as a fat replacer for developing low-fat meat products.

Another researcher also investigated the use of nanobacteria cellulose (NBC) in low-lipid and low-sodium meat sausages (Marchetti *et al.,* 2017). The bacterial nanocellulose (NBC, 0–0.534 g of dry NBC/100 g batter) was added into meat emulsions containing pre-emulsified high oleic sunflower oil. The yield, water content, water activity, water holding capacity, colour, texture, rheological characteristics, microstructure, and shelf life of the meat sausages were analysed. Apart from that, NBC with the value of 0.267

g of dry NBC/100 g batter showed excellent water-binding and texture (e.g., hardness, cohesiveness, and chewiness) properties. Value of NBC above 0.267 g of dry NBC/100 g batter showed negative impacts on physical and chemical attributes such as texture, rheological characteristics and water content of the sausages produced. These attributes. As such, NBC can be used as one of the fat replacers for producing low-fat meat products.

3.3 Nanocellulose in mayonnaise

Mayonnaise is a semi-solid solution manufactured with edible oils, egg yolks, salt, vinegar, thickening agents, and flavouring materials. Commercial mayonnaise has mostly 70–80% fat content, and it is easily produced as low-fat versions for conscious consumers. The "light" mayonnaise products contain about 36% fat and starches, cellulose gel, or other ingredients as fat replacers, which stimulate the mayonnaise's texture (Aylin *et al.*, 2018). It can help consumers reduce their fat intake to the recommended levels. Many research has demonstrated that nanobacteria cellulose (NBC) has various essential applications in a variety of food formulations because of its unique properties and structures that distinguish it from other forms of cellulose, such as high polymer crystallinity, high degree of polymerization, high purity, high tensile strength, solid biological adaptability, and high-water absorption and retaining capacity (up to 700 times its dry weight).

Research on formulating low-fat mayonnaise was conducted to investigate the addition of nanofibrillated cellulose (NFC) in the formulation (Golchoobi *et al.*, 2016). In this study, the author has investigated the interaction between nanofibrillated cellulose (NFC) with guar gum and carboxymethylcellulose (CMC) in low-fat mayonnaise. It was found that the stability of mayonnaise containing NFC or NFC/CMC was observed to be lower than the control sample containing NFC/guar due to its viscodifying effect, which presented higher stability levels. Moreover, no significant difference was observed between the taste of treated mayonnaise and the commercial control sample. These results prove that NFC can be used as a fat replacer to produce low-fat mayonnaise. The positive effect of NFC on the mouthfeel features in organoleptic properties was also confirmed. They concluded that the addition of NFC and guar gum to low-fat mayonnaise had improved the product's physicochemical, rheological, and organoleptic characteristics.

4.   Toxicology analysis of nanocellulose

Cellulose has been used widely as a thickener and filler in foods and drugs, and it has been categorized as "generally regarded as safe" (GRAS). However, although nanocellulose has been reported to provide many potential applications to improve food property and safety, it has not been designated as GRAS. Thus, investigating the toxicology of ingested nanomaterials is of great importance. Manufacturers have adopted a proactive approach the safety to demonstrate NCC safety toward responsible commercialization. As part of the safety demonstration, in vivo and in vitro testing approaches were commissioned side-by-side with conventional cellulose, safely used in food for decades. Silva *et al.* (2019) have investigated the toxicology of nanocrystal cellulose and nanofibrillated cellulose in vitro intestinal epithelium and in vivo rat models. In vitro studies employed a gastrointestinal tract simulator to digest two nanomaterials: nanofibrillated cellulose and nanocrystal cellulose at 0.75% and 1.5% w/w, in a fasting diet as well as in a standardized food model based on the average American diet. It was reported that no significant differences in haematology, serum markers or histology were observed between controls and rats given nanofibrillated cellulose suspensions. It is also reported ingested NC has little acute toxicity and is likely non-hazardous when ingested in small quantities.

Furthermore, Ede *et al.* (2020) also investigated nanocrystalline cellulose's toxicology using an in vivo and in vitro approach. They investigate the physical, chemical and biological of NCC, together with conventional cellulose material. The testing strategy adopted a tiered approach to complete a full physical, chemical, and biological characterization of these novel cellulose forms side-by-side to conventional cellulose (CC) material. In this study, three control groups fed diets with 2, 3, and 4% CC. Any adverse effects resulting from higher or lower dietary fibre intake could be distinguished from effects related to the NCC test material itself. They reported that the 90-day subchronic toxicity test found no adverse effects from oral NCC or CC exposure in rats up to 4% of the diet. The simulated gastrointestinal and lysosomal digestion of NCC and CC did not significantly change their physical or chemical characteristics. This suggests that both NCC and CC remain relatively unchanged physically and chemically as they undergo digestion. Humans lack digestive enzymes capable of breaking down cellulose; several absorptions, distribution, metabolism, and excretion studies have shown that it is generally 100% excreted in the faeces. Hannukainen *et al.* (2015) are also reported that nanocrystalline cellulose is neither genotoxic nor immunotoxic under, compared to non-nano sized microcrystalline cellulose can induce an inflammatory response.

5. Challenge and future perspectives

Cellulose and nanocrystalline cellulose provide a vital role in the food industry due to their excellent properties. NCC can be used as an alternative fat replacer. All previous work discussed in this review paper showed that NCC is ideal to be used as a food additive in enhancing the quality of food products. However, nanocrystalline cellulose extraction from agricultural by-products remains a challenge because of the substantial aggregation of non-cellulosic materials, such as lignin and hemicellulose, that need to undergo chemical treatment. Acid hydrolysis is usually used to remove non-cellulosic compounds from agricultural waste. This process might produce a high amount of acid in wastewater due to the washing step. Besides that, high energy consumption can be observed during nanofibrillated cellulose isolation using the mechanical process. An extended reaction time is required for the enzymatic hydrolysis of nanocrystalline cellulose. Furthermore, the properties of nanocrystalline cellulose produced should be improved via crossover with traditional methods. The application of NCC in the food industry needs further focus on its nutritional properties, storage stability, and consumer acceptability of the products.

Conflicts of interest

All authors have declared no conflict of interest in preparing this article.

Acknowledgements

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