Cereal Starch Nanoparticles- A Prospective Food Additive: A Review

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Starch is one of the most abundant biopolymers in nature and is typically isolated from plants in the form of micro-scale granules. Raw starch has limited applications due to its innate disadvantages such as poor solubility in cold water, tendency to retrograde and high viscosity once it is gelatinized. Therefore, some degree of modification is required to enhance its functionality. Starch nanoparticle is one of the products of such modification. Chemical, enzymatic, and physical treatments are used for the preparation of starch nanoparticles and to study their granular and molecular structures. Characterization of starch nanoparticles on the size distribution, crystalline structure, and physical properties in relation to the starch sources and preparation methods can be done using various characterization tools e.g. Scanning Electron Microscopy, Transmission Electron Microscopy, Atomic Florescence Microscopy, etc. Starch nanoparticles can be used as a food additive as it has adverse range of uses in food such as emulsion stabilizer, fat replacer, Thickener or rheology modifier etc.

Introduction

Starch is a natural, renewable, and biodegradable polymer produced by many plants as a source of stored energy. It is the second most abundant biomass material in nature. It is found in plant roots, stalks, crop seeds, and staple crops such as rice, corn, wheat, tapioca, and potato (Buleon *et al* 1998). The starch industry extracts and refines starches by wet grinding, sieving, and drying. It is either used as extracted from the plant and is called "native starch", or it undergoes one or more chemical modifications to reach specific properties and is called "modified starch". Worldwide, the main sources of starch are maize (82%), wheat (8%), potatoes (5%), and cassava (5%) from which tapioca starch is derived (Angellier *et al* 2004). In 2000, the world starch market was estimated to be 48.5 million tons, including native and modified starches. The value of the output is worth €15 billion per year, explaining the industrialists and researchers seeking new properties or high value application(http://ec.europa.eu/agriculture/eval/reports/amidon/chap1.pdf).

It consists of mainly two glucosidic macromolecules: amylose and amylopectin. In most common types of starch the weight percentages of amylose range between 72 and 82%, and the amylopectins range from 18 to 28%. However, some mutant types of starch have very high amylose content (up to 70% and more for amylomaize) and some very low amylose content (1% for waxy maize). Amylose is defined as a linear molecule of glucose units linked by (1-4) R-D-glycoside bonds, slightly branched by (1-6) R-linkages. Amylopectin is a highly branched

polymer consisting of relatively short branches of R-D-(1-4) glycopyranose that are interlinked by R-D-(1-6)-glycosidic linkages approximately every 22 glucose units (Dufresne *et al* 2007). Along with recent interest on nanomaterials, some researchers utilized starch nanoparticles (SNPs) as a filler in composites, and found that the incorporation of SNPs improved not only the mechanical properties but also the biodegradability of the composites. In addition, the SNPs have been reported to be applicable in other areas, such as foods, cosmetics, and pharmaceuticals. However, the industrial utilization of SNPs is limited until now,and only two applications (BioTRED and Eco-sphere TM) have been reported. One of the applications is called BioTRED (Novamont,Italy), a tire developed in collaboration with an American company(Goodyear, USA). The nanoparticles that had been derived from corn starch were used to replace a part of the carbon black and silica in tire. The incorporation of the SNPs provided an improvement in relining resistance of the tire with sphere TM, a starch-based biolatex that substitutes for the oil-based latex. It has been used as a replacement for petroleum-based coating and binders for paper and paperboard. In this review, various processes for the preparation of SNPs using chemical, enzymatic, and physical treatments were introduced, and granular and molecular structures of SNPs were discussed. The nanoparticle preparation from starch can be classified into "top-down" and "bottom-up" methods according to the preparation scheme.

Characterization of the SNPs on the size distribution, crystalline structure, and physical properties was discussed in relation to the starch sources and preparation methods. Finally, potential utilization of SNPs was discussed based on their physical characteristics. In most published researches, the SNPs were focused on their use as reinforcing polymers in composites. Therefore, potential applications of SNPs are discussed.

Starch- Granular Structure and Properties

Starch is mainly composed of amylose and amylopectin which are different in chain structure (shown in Fig 1.). Amylose is defined as a linear molecule of (1→4) linked α-D-glucopyranosyl units, but it is today well established that some molecules are slightly branched by (1→6)-α-linkages. Amylopectin is the highly branched component of starch: it is formed through chains of α-D-glucopyranosyl residues linked together mainly by (1→4) linkages but with 5–6% of (1→6) bonds at the branch points. The starch molecules are biosynthesized, and are densely packed in granules with dimensions ranging from 1 to 100 µm (See table 1). In contrast to tubers and legume starches, cereal starches are characterized by the presence of monoacyl lipids (free fatty acids (FFA) and lysophospholipids (LPL) in amounts positively correlated to amylose content (Morrison *et al* 1993).

Starch granules when viewed under the optical microscope have a characteristic layered structure, due to the feature called as 'growth rings' (Gallant *et al* 1997) (As shown in Fig.2).This is the result of multiple concentric shells (or lamellae) of increasing diameter extending from the hilum towards the surface of granules (like the layers of an onion). These growth rings represent (periodical) diurnal deposition of starch as originally defined by Robin *et al* (1974), usually based on data generated by acid or amylase treatment of granules.

The alternating crystalline and amorphous lamellae organize into larger,more or less spherical ,structures, which has been termed 'blocklets', in a diameter range of 20-500nm, depending on the starch origin and location in the grantule. Furthermore, recently it was revealed that the semicrystalline lamellae were densely packed with a number of 'blocklets' having diameter from

20 to 500 nm depending on the botanical source of starch and location in granule (Gallant *et al* 1997). The blocklets have an asymmetric structure with an axial ratio of 2 or 3:1 (Tang *et al* 2006), size is smaller in the semicrystalline shells than in the crystalline shells. Crystalline blocklets are evident in partly hydrolysed starch granules in the form of residues comprising small, more or less spherical blocks that are tangentially arranged and tightly packed. Packing is in tiny crystalline sheets (approx. 5nm thick), which are stacked tangentially to the granule surface.In the hard parts of the shell, the blocklets may be 100nm in diameter, whereas they are only 25nm in soft parts. The tiny blocks are larger in B and C- type granules than in A-type granules. In B-type granules much larger blocklets are superimposed at the peripheral level to a depth of 10μm (Oates 1997).

 (-5 nm thick), which are stacked tangentially

Starch nanoparticles : Preparation

The preparation of starch nanoparticles may be classified on the basis of their mode of preparation ie. Chemical Physical and Enzymatic or their combinations. In this review these preparation methods has been explained. However the preparation of Starch nanoparticles has been classified into top down and bottom up approach. In "top-down" process, through a breakdown of larger particles, nanoparticles can be produced from structure and size refinement. In "bottom-up"process, nanoparticles can be prepared from a buildup of atoms or molecules in a controlled manner that is regulated by thermo-dynamic means such as self-assembly (Tan *et al* 2009). Although a few studies have been reported on the basis of the "bottom-up" approach such as nanoprecipitation (Ma *et al* 2008), most studies reported the preparationof SNPs employed "top-down" methods.

CHEMICAL METHODS

Acid hydrolysis

Acid hydrolysis is a simple and ready to control method, this is the reason it is widely used in preparation of starch nanoparticles. There are two ways of monitoring hydrolysis of starch granules: the content of soluble sugar in solution (phenol–sulfuric acid) and the recovery of insoluble starch residues. Most of the starches exhibit a two-stage hydrolysis pattern: fast initial hydrolysis followed by slow subsequent hydrolysis (Jayakody and Hoover 2002). However, some authors distinguished three stages of the acid hydrolysis: rapid, slow, and very slow (Angellier *et al* 2005) . The initial stage of acid hydrolysis is thought to be the hydrolysis of the amorphous parts within starch granules, whereas the slow stage is attributed to the erosion of the crystalline regions (Kainuma and French 1971). The preparation of these starch nanoplatelets required a long duration of hydrolysis (40 days of treatment) and the recovery yield from the starch granules were relatively low(0.5 wt%). Moreover, the nanoplatelets tended to aggregate to form microscale particles.

Angellier *et al* (2004) employed the hydrolysis using sulfuric acid for the preparation of starch nanoparticles, instead of the hydrolysis using HCl. The H2SO4 hydrolysis shortened the preparation time, and increased the yield of SNPs. The formation of sulfate–ester linkages on the surface of nanoparticles during the hydrolysis should limit the flocculation of nanoparticles and thus produce a nanosuspension with increased stability (Angellier *et al* 2005). This effect was confirmed by comparing these dimentation rate of the suspensions obtained by using each acid.The stability of nano suspensions also depends on the dimension of the dispersed particles

and polydispersity (Liu *et al* 2009).

The crystalline residues remaining after a prolonged acid hydrolysis consisted of agglomerated particles of a few tens of nanometers in diameter (Dufresne *et al* 1996). In 2003, Putaux *et al* (2003) observed an edge which was formed by the association of amylopectin side-branches, on view of the lamellae under the TEM. By subjecting native waxymaize starch to a hydrochloric acid hydrolysis (2.2 N HCl) for 2 weeks, it was found that a lamellar arrangement had been disrupted to some extent. In other words, a certain number of α (1 $\rightarrow$ 6) bonds that located in the amorphous regions between the crystalline lamellae were hydrolyzed. Because the branching points located in the interlamellar areas were more readily hydrolyzed, the insoluble residues were fairly well individualized. Indeed, the individual residues could be observed after 6 weeks of hydrolysis under a microscopy. The hydrolysis residues were crystalline nanoplatelets of approximately 6–8 nm thickness, 20–40 nm length, 15–30 nm width (Putaux *et al* 2003).

Nevertheless, the acid hydrolysis is an energy-intensive process for the production of SNPs and recovery of SNPs is low. The salts produced by neutralizing, and soluble sugars should be carefully removed from the reaction medium, with no or little loss of SNPs. Obtaining uniform SNPs with a high yield is still a task in the process using acid hydrolysis.

The suspension of the particles remains as greater stability as the particles are obtained in smaller size and higher uniformity, The sulfate ester bonds on the starch surface may decrease the thermal stability of the nanoparticles, which could negatively affect their use in composites. Thermal stability of the SNPs suspensions could be enhanced by adding a small amount of ammonia (Chang *et al* 2009). Most of the acid hydrolysis works were carried out with waxy

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maize starch. However, other sources of starch having different amylose contents and crystallinity were also tested in a recent study (Kim *et al* 2012).

Emulsion-crosslinking

Synthesis of SNPs by emulsion-crosslinking technique was done. It involves the dispersion containing hydrophilic natural materials such as starch and cross-linkers in oil phase with the presence of emulsifiers. The small particles can be generated in emulsion through cross-linking reaction. These particles obtained from emulsion-cross linking approach were relatively big in micro-scale. Fang *et al (*2008), and Franssen and Hennink (1998) observed starch microspheres with the average diameter of 19 μm and ranging from 2.5 to 25 μm through this emulsion-crosslinking method. In order to reduce the size of these particles, emulsion should contain nanoscale droplets, which is generally referred to miniemulsion, submicron emulsion or nano emulsion. It is because the emulsion droplets maintain their shape and size within the dispersed phase. Zhou *et al* (2014) attempted to reduce the particle size using ionic liquid-in-oil (IL/O) microemulsion system instead of traditional water-in-oil (W/O) emulsion approach. By substituting the water phase using 1-octyl-3-methylimidazolium acetate, starch nanoparticles with an average diameter of 96.9nm were obtained. Another study suggested the possiblity of production of SNPs through in a water-in-ionic liquid (W/IL) microemulsion system (Zhou *et al* 2014), which was an efficient and environmentally friendly approach because no toxic organic reagents such as cyclohexane were used as oil phase. Miniemulsion may be produced by specially designed devices which provide sufficient mechanical energy such as high pressure homogenizers, which ultimately increases emulsification efficiency and reduces the cost with

less amount of surfactant. High-pressure homogenizer was employed by Shi *et al* (2011) at pressures ranging from 10 MPa to 60 MPa , miniemulsion displayed a good sphericity in shape of nanoparticles and a comparatively uniform size distribution. The size of the particles varied according to the process parameters including surfactant content, starch concentration, water/oil ratio, and homogenization pressure and cycles.

Nanoprecipitation

In Nanoprecipitation process there is the successive addition of a dilute solution of polymer to the solvent which leads to the polymer polymer precipitation on nanoscale. This method is based on the interfacial deposition of polymers following the displacement of a semipolar solvent which is miscible with water from a lipophilic solution. This approach has many advantages: submicron particle sizes with narrow size distributions can be obtained as well as large amounts of toxic solvents and external energy sources are avoided, and (Mathew and Dufresne 2002). Tan et al. prepared nanospheres using acetylated waxy maize starch by a nanoprecipitation process (Ma *et al* 2008). A dispersion of waxymaize starch acetate in acetone was added by distilled water dropwise to form nanospheres, and acetone was vaporized from the aqueous suspension. The size of the obtained nanospheres depends on the concentration of polymer in the acetone. As the concentration of the starch in the acetone increases from 1 to 20 mg/mL, the mean diameter increases from 249 to 720 nm.

Similar studies were conducted by the group of Ma *et al* (2008) .They prepared SNPs by precipitating starch paste solution with ethanol as the precipitant and then modified the obtained SNPs using citric acid (CASN). Gelatinizedation SNPs were gradually precipitated,when ethanol

was delivered dropwise to a starch solution. Therefore, A-type crystalline structure of cornstarch was destroyed by the gelatinization, and the SNPs exhibited the VH-type crystalline structure. However, most of the crystals of CASN had disappeared. The CASN ranged in size from approximately 50–100 nm and because of the cross-linking by citric acid it is not gelatinized during the processing of the nanocomposites.

Physical treatments

High-pressure homogenization

Liu *et al* (2009) developed versatile and environmental friendly approach for the isolation of SNPs using a high pressure homogenization technique (As shown in Fig. 3). High-pressure homogenization through a microfluidizer is based on the principle of manipulation of a continuous flow of liquid through microfabricated channels. Actuation of the liquid flow is implemented by external mechanical pumps, external pressure sources, integrated mechanical micropumps,or electro kinetic mechanisms (Hale *et al* 2001). In a system that has an electronic-hydraulic intensifier pump, the product stream accelerates to high velocities, creating high shearing within the product stream. When 5% starch slurry (high amylose corn starch, Amylogel TM was passed through a specially designed microfluidizer 20 times under a pressure of 207 MPa, the particle size of starch granules reduced from 3–6 µm to 10–20 nm (Song *et al* 2011).

This size reduction was attributed to a result of the breakage of the hydrogen bonding inside the large particles by the mechanical shear forces. However, it was also observed that partial or

complete destruction of the crystalline structure occurred. In addition, because only low concentration of starch slurry could be processed for the homogenization, the recovery yield for each process is low (Song *et al* 2011).

Ultrasonication

Ultrasound describes the sound waves at a frequency that is above the normal human hearing range (>15–20 kHz). These are produced with transducers (either piezoelectric or magnetostrictive) that create high-energy vibrations. These vibrations are then amplified and transferred to a sonotrode or probe that is in direct contact with the fluid (Shown in Fig.4). The effect of ultrasound on starch depends on many parameters such as properties of starch dispersion sonication power and frequency, temperature and time of the treatment. (Zuo *et al* 2009). Another important factor is called as cavitation which includes the formation of bubbles of gases in the suspension medium, that bombard starch granules before they collapse (Tomasic and Zaranyika 1995). Rapid collapsing bubbles could also arise the shear force that may break the polymer chains. Ultrasonication of starch suspension was also introduced as a process for the preparation of SNPs (Haaj *et al* 2013). In this study, an aqueous suspension of waxy maize starch (1.5% solids) was treated by ultrasonication in a water bath (8∘C) for 75 min.

Low temperature was maintained during ultrasonication so that water molecules could not diffuse inside the amylopectin chains, and no plasticization of the amylopectin phase was likely to take place. The authors claimed on the basis of SEM observations that mechanical collision along along with high shear force brought about a progressive erosion of the starch particles starting from the surface, which underwent further fragmentation until a limiting size between

30-100nm was reached.

Compared with the common acid hydrolysis, this process offered the advantages of being rapid and easy to implement as there is no the need to undertake repeated washing treatment because no chemical reagent is used. The recovery yield maybe 100% because no further purification is needed after the SNPs are produced in solution. It was found on the basis of wide angle X-ray diffraction analysis that ultrasonication seriously disrupted the crystalline structure in the starch and lead to nanoparticles that had a low crystallinity or an amorphous structure (Haaj *et al* 2013).

Reactive extrusion

Reactive extrusion (REX) is a process combining the mass and heat transport operations with simultaneous chemical reactions taking place inside the extruder for the purpose of modifying the properties of existing polymers or for producing new ones (Tzoganakis and Zhu 2012). This method includes the loading of the premixed starch and plasticizer in a twin screw extruder, and addition of reversible crosslinkers, such as glyoxal. . Starch Nanoparticle formation via this method has also been investigated (Giezen *et al* 2000).The SNPs Produced by the method through crosslinking is to considered to be Regenerated starch nanoparticles (Lai *et al* 1990). Starch is subjected to relatively high pressure (up to 103psi), heat and mechanical shear forces during the extrusion (Lai *et al* 1990). . It was confirmed that starch particles with a size of less than 400 nm could be produced by using reaction extrusion (Giezen *et al* 2000). The hydrogen bonding between starch chains can be broken due to the high shear force and temperature. As a result, significant structural changes such as gelatinization, melting, and fragmentation might

occur There was an incomplete gelatinization due to limited water content during the extrusion. But at such high extrusion temperature, starch granules soften and partially melt, and become mobile, which were physically torn apart by the shear force that allows water to transfer into the interior of starch granules. The SNPs prepared by this method had very low viscosity but was stable for more than 6 months (Giezen *et al* 2000). Similar to other physical treatments, mechanical damage of starch crystals was also caused by a high energy level of extrusion.

Gamma irradiation

Gamma irradiation has been suggested as a rapid and convenient modification technique that fragments large molecules by cleaving the glycosidic linkages (Kim *et al* 2014). Gamma irradiation can generate free radicals which can hydrolyze chemical bonds, thereby producing smaller fragments of starch called dextrin. Lamanna *et al* 2013 prepared SNPs by gamma irradiation and compared with the nanoparticles obtained from acid hydrolysis . SNPs having a size of approximately 20 and 30 nm were obtained by applying a dose of 20 kGy from cassava and waxy maize starches with an irradiation rate of 14 kGy/h), respectively (Lamanna *et al* 2013). Thermal characterization by a simultaneous instrumental analysis revealed that the SNPs obtained from irradiation were more susceptible to thermal degradation than the parent native starch. It suggested that SNPs have a large number of hydroxyl groups on their surface where the thermal degradation starts, similarly to the thermal properties for SNPs obtained by acid hydrolysis. However, the nanoparticles from gamma irradiation with starch displayed amorphous XRD pattern similar to the SNPs obtained from other physical treatments. The authors explained that the transformation to amorphous structure was caused by the heat treatment (85°C) prior to

the gamma irradiation, which was necessary to obtain stable dispersions.

COMBINATIONS

Enzymatic treatment followed by acid hydrolysis

The SNPs preparation could be done within a reduced time by using the combined procedure (LeCorre *et al* 2011). By pretreatment of starch with glucoamylase the time of acid hydrolysis could be decreased effectively. The amorphous regions become hydrolysable more readily as this pretreatment created the pathways for the acids to diffuse inside the granules. At a same extent of hydrolysis, the SNPs from the pretreated starch could be obtained at a higher yield. Only drawback is the larger size (145 nm) compared to those obtained from the non-treated starch (usually between 50 and 100 nm). The acid hydrolysis that normally required 5 days was obtained after only 45 h (yields of 15%). The increase in the size is possible due to the tendency of acid hydrolyzed nanopartices

Combination of hydrolysis and ultrasonication

The SNP preparation by acid hydrolysis alone took several days. However, the acid hydrolysis for 2 days was sufficient to remove most of the amorphous regions in starch (50–70% of starch). Considering that, the relative crystallinity of waxy maize starch was reported to range from 30% to 50% (Jayakodi and Hoover 2002).

Kim *et al*. (2013) reported the preparation of SNPs from waxy maize starches by a combined process of acid hydrolysis (2 days at 40∘C) and subsequently ultrasonic treatment (60% vibration amplitude, 3 min) was applied to the redispersed suspension of the large microparticles of starch

hydrolyzates, which had been recovered by a mild centrifugation(500 rpm,10 mins). By this facile process, the microparticles could be completely transformed to nanoparticles. However, recovery yield of the crystalline SNPs becomes low (less than30%), as the ultrasonication often disrupts the crystalline structure of starch.

In the study by Kim *et al* 2013, a low temperature (4∘C) was applied to facilitate the formation of new crystalline structure in the SNPs by chain association, the association of the starch chains and minimizing the crystallinity disruption .The starch hydrolyzates, obtained after 6 days of acid hydrolysis, were more resistant to the subsequent ultrasonication than those obtained after 2 or 4 days, regardless of the hydrolysis temperature. The low temperature acid hydrolysis in the combined process was an effective process for the mass production of crystalline SNPs. In addition, the combination of acid hydrolysis and ultrasonication increased the recovery yield of SNPs (15% vs 78%) compared to the ordinary process with acid hydrolysis alone.

Enzymatic hydrolysis and recrystallization

A green and facile method for obtaining nanoparticles from proso millet starch was suggested (Sun *et al* 2014). In this study, starch was cooked and treated by pullulanase. The solution was stored at 4℃ for starch to recrystallize and SNPs with a size range between 20 and 100 nm can be isolated by centrifugation. The study showed that debranching with pullulanase facilitated the recrystallization of starch chains. This approach has the advantage of being quite rapid and presenting a higher yield (approximately 55%) as compared to the conventional acid hydrolysis. This process also offers the advantage that there is no need of giving washings as no chemical reagent is added during the preparation process.

Enzymatic treatment

An enzymatic hydrolysis of waxy rice starch using α-amylase for 3 h induced the fragmentation of starch granules with selective dissolution of the amorphous regions (Kim *et al* 2008). The volume-based size distribution of the starch exhibited two major peaks at 0.5 and 3.6 μm and a shoulder at 0.1 μm after the enzymatic hydrolysis. However, the average diameter of the particles remained fairly large (approximately 500 nm) (Table 2), which was greater than the value of the blocklets (20–500 nm) reported by Gallant *et al* (1997). The authors assumed that the enzymatic hydrolysis of the amorphous regions induced the fragmentation of the granules, and a portion of the blocklets could be separated. Combination of both enzymatic and acid hydrolysis has been used for the preparation of SNPs.

Not much research has been performed on the enzymatic process for the preparation of SNPs, compared to the acid hydrolysis.

Complex formation followed by enzymatic hydrolysis

In 2009, Kim et al. developed a new process to obtain SNPs based on complex formation between amylomaize starch and n-butanol and subsequent enzymatic hydrolysis (Kim *et al* 2009). First, a dilute starch solution was placed in the upper compartment of a glass filtration apparatus (Millipore, Billerica, MA, USA) and allowed to gravimetrically pass through a membrane filter (PTFE, 10 μm pore size, 47 mm diameter, Millipore, MA, USA) into the bottom compartment, which was filled with n-butanol. The filtration apparatus was kept at 70°C in a

convection oven for 6 days. The starch–butanol complex was isolated as the precipitate and subjected to α-amylolysis. Because the amylose-butanol complex that formed in the butanol layer contained a large portion of amorphous matrices, selective removal of these was required to isolate the nanoparticles. The subsequent enzymatic treatment made the SNPs spherical or oval shape with diameters of 10–20nm (Table 2). However, there was a significant loss by hydrolysis (85–90%) of the starch, and thus the overall yield of the nanoparticles was very low.

Characterization of SNPs

Morphology

Morphological characteristics of SNPs depend on preparation method as well as the botanical source. Starch nanoparticles were prepared from five starches of different origins (e.g., normal maize, high amylose maize, waxy maize, potato, and wheat) and their morphologies were compared (LeCorre *et al* 2011). It was concluded that the morphology of SNPs appeared to be related to the crystalline structure of original starches. Overall, the nanocrystals produced from A-type starches (e.g., waxy maize, normal maize, wheat starch) rendered square-like particles, whereas those from B-type starches (e.g., high amylose maize, potato) produced round-shaped particles (See fig.5). This difference in shape indicates that the blocklets between the A and B-type starches have different morphologies. The differences in the arrangement of amylopectin double helices in crystal lattices might induce the formation of different blocklets.

In another research by Kim *et al* 2012, starch nanoparticles were prepared from various starch

sources and their morphology was characterized through TEM. It was concluded that regardless of their starch origin, nanoparticles had round or oval shapes.Also size of starch nanoparticles from B- type and C-type starches was larger than A-type starches.

Although there are some differences in the morphology of SNPs, it is obvious that the SNPs which are obtained from the hydrolysis form microscale agglomerates due to their tendency of self-aggregation. This can be explained by the presence of a large number of hydroxyl groups on the surface of SNPs, which readily participate in the formation of hydrogen bonding or van der Waals attraction between SNPs (Wei *et al* 2014).Because of this aggregation behavior industrial application of SNPs is limited. The average size of the starch nanoparticles obtained by different methods are shown in Table 2.

Crystallinity

The changes in the crystalline structure of starch during the production process of SNPs have been focused by most of the studies. The relative crystallinity (RC) of the SNPs to the crystallinity of original starch was assumed to be positively related to the recovery yield of the SNPs. LeCorre *et al* (2011) investigated the influence of the botanic origin of starch on the crystallinity following the method of Nara and Komiya (1983) using an X-ray diffraction analysis. The RC of the SNPs increased as compared to the native starch because the amorphous regions were selectively removed by the acid hydrolysis. Among the maize starches containing

different amounts of amylose, with the decrease in the amylose content the RC of the SNPs from maize starches increased (35% and 48% for high amylose maize and waxy maize starch, respectively).But no significant differences were observed in the degree of crystallinity of their SNPs (e.g., 42% and 43% for normal maize and potato starch, respectively) when the starches having similar contents of amylose (21–28%) with different botanic origins were compared. It was demonstrated in this study that amylose content in the starch was the most important parameter in determining the degree of crystallinity of SNPs. Similar results were reported by Duan *et al* 2011 . The RC of waxy maize SNPs were 48% and 63% after 4 and 6 days of acid hydrolysis, respectively. After 10 days of hydrolysis, the RC of the SNPs could be increased up to 79%, but its yield became very low (10%).

Rheological Properties

Pasting behavior of SNPs was different as compared to the native starch (Ma *et al* 2008).Native corn starch granules swelled when heated in excess of water which resulted in disruption of granule structure and thus increase in viscosity. As SNPs were prepared by acid hydrolysis, the pasting viscosity was much lower than of native starch. However with the increase in amount of SNPs, the pasting viscosity increased. Also with the increase in temperature viscosity of SNPs increased continuously till it reached 95℃.When the temperature decreased from 95 to 50℃, the viscosity of SNPs increased again.It was revealed by viscograms that SNPs does not show rapid increase in viscosity as compared to native starch, which may be due to the reduced size.

 Shi *et al* 2012 investigated the rheological properties of the suspensions containing starch nanoparticles. The continuous shear viscosity tests, temperature sweep tests, the frequency

sweep and creep recovery tests were carried out.The suspensions containing freeze dried starch nanoparticles showed higher apparent viscosity within shear rate range(0.1-100 s^{-1}) and temperature range (25-90°C).The suspensions with vacuum freeze dried nanoparticles were having more shear thinning compared to those containing spray dried starch nanoparticles. In addition to these the suspension with vacuum freeze dried nanoparticles had stronger elastic structure, whereas those with spray dried nanoparticles had more stiffness and greater tendency to recover from deformation.

Molecular composition

The molecular properties of SNPs have been rarely studied (Kim *et al* 2012, Angellier *et al* 2009).The molecular composition of the nanoparticles prepared from waxy maize starch was reported by Angellier *et al.* (2009).Waxy maize SNPs, which consisted of double helices containing α-(1→6) linkages between the parallel strands, were progressively degraded into low molecular weight oligosaccharides when they were subjected to debranching enzymes (isoamylase and pullulanase) followed by amylase. Eight different dextrins were found in the SNPs prepared by the enzymatic treatment, and classified into two major groups: A and B populations.The population A consisted of multi branched dextrins with an average degree of polymerization of 31.7 while population B contained at least one branch and average degree of polymerization was 14.2. The mole percent of branched chains of Population A and B were similar.

Thermal transition properties

The characterization of thermal transition behavior of SNPs has been done by differential scanning calorimetry (DSC). the endothermic melting transition peak of the SNPs, prepared by acid hydrolysis, was observed in broadened ranges as compared to the native starch,. This observation was regardless of the processing methods for SNPs (Kim *et al* 2012, Lamanna *et al* 2013, Kim *et al* 2013, LeCorre *et al* 2013). However, melting temperatures and enthalpy depended on the parameters for the SNP preparation and the botanical source of starch. The broad melting range of SNPs could be attributed to the heterogeneity of the SNPs in which amorphous and crystalline phases were in mixture (Lamanna *et al* 2013). It was reported by Kim *et al* (2013) that by changing the preparation conditions such as hydrolysis temperature and time, SNPs could be obtained with different melting characteristics.

APPLICATIONS

Non Food applications

Drug carrier and implant material

More recently, SNPs also have received a great attention in this application. Starch is nontoxic, biodegradable, and biocompatible polymer, thus it is an excellent carrier for drug delivery. Nanoparticles have the ability to deliver an ample range of molecules to different locations in the body for sustained periods of time. A higher intracellular uptake of nanoparticles has been reported compared to micron-sized particles. Nanoparticles can access a variety of biological tissues because of their tiny size and mobility. Indeed, the nanoparticles larger than 230 nm in

size could congregate in the organ especially in the spleen due to the capillary size of this organ (Kreuter 1991). Starch has been used as a delivery carrier for anti-cancer drugs (Xiao *et al* 2006) and transdermal drugs (Santander-Ortega *et al* 2010). It was used as a carrier for phenethylamines, acetylsalicylic acid (Aspirin), and estrone. Chemically modified starches or SNPs were also reported to be used in sustained drug delivery systems. For example, a cross-linked high amylose starch was used as a matrix for the controlled release of contramid. Propylated SNPs that had been loaded with different types of drug (flufenamic acid, testosterone, and caffeine) showed enhanced effectiveness in the permeation through human skin (Santander – ortega *et al* 2010). More recently, curcumin has shown to have antitumor effects in many cancer cell lines and the clinical effects of curcumin are being studied in human clinical trials and animal models on various conditions and numerous myeloma Curcumin loaded starch nanoparticles exhibited enhanced solubility in aqueous solution as compared to free curcumin (Chin *et* al 2014). Under optimum conditions, curcumin loaded starch nanoparticles with mean particles size of 87 nm and maximum loading efficiency of 78% were achieved. Curcumin was release out from starch nanoparticles in a sustained way under physiological pH over a period of 10 days (Chin *et al* 2014).

Wastewater treatment

Chemically modified SNPs can be used as adsorbents for the removal of aromatic organic pollutants from water (Alila *et al* 2011).Currently, activated carbon is the most commonly used adsorbent for the removal of toxic organic substances from water (Philip *et al* 2004). However, in spite of its prolific use, activated car-bon is expensive. A synthetic polymer may also be used,

but there is a growing interest in developing renewable and low-cost alternatives. Biopolymer-based materials may be the most attractive adsorbent for wastewater treatments. A recent study by Morandi et al. showed the large absorption potential of polystyrene-modified cellulose nano whiskers for the removal of aromatic organic molecules from water. Alila *et al* (2011) first reported the potential use of starch nanoparticles as adsorbent after modified by grafting with stearate. Compared to native biopolymers, the nanoparticles derived from those have increased efficiency and capacity due to the increased surface area, as proved by the cellulose nanowhiskers (Morandi *et al* 2009).

Thermo-responsive conducting

Valodkar *et al* (2010) synthesized a crosslinked SNPs with 1,4-hexamethylene di isocyanate which were insoluble and sufficiently reactive for the use in synthesis of nanocomposites. It is well-known that the aliphatic chain of six methylene groups in HMDI is highly flexible among various isocyanates (Valodkar and Thakore 2010).The SNPs behave as nanofillers and crosslinkers in the synthesis of PPG-based polyurethanes (PU). The highly crosslinked and nanofilled polymeric systems are likely to exhibit a good electrical conductivity with a high rigidity. This effect is further enhanced by an internal plasticizing effect of the nanoparticles.

papermaking and paper coating

The SNPs can be used as a binder in papermaking and paper coating. The cooked starch has been widely used as paper-making additives. The retention of the cooked starch on the paper matrix is based on the absorption of starch. Thus, the absorbed amount of starch depends on the saturation

of absorption on cellulose substrates. Another problem is the high viscosity of the starch paste after cooking of raw starch, which might cause operational problems. It was reported that SNPs could be used instead of cooked starches to improve the performance as binder in paper (Bloembergen *et al* 2008). With the addition of SNPs, the viscosity of the paste can be substantially reduced, whereas the binding capability can be increased.

In composites

"Composites" are materials that can be defined as combination of two or more materials that result in better properties than those of individual components used alone. Basically a composite is composed of two types of components: the matrix which is to support and protect the filler materials, transmit and distribute the applied load to them and the mentioned fillers, which are the stiffer and stronger components that reinforce the matrix. Furthermore, "Nanocomposites" mean polymeric composite materials with reinforcement of nano-sized particles (Nair and Dufresne 2003). The advantages of these nanocomposite materials over conventional composites are their superior mechanical, barrier, and thermal properties at low levels as well as their recyclability, transparency and low weight (Oksman *et al* 2006).As starch is a biodegradable and nontoxic polymer, it has been widely used in non-food applications including papers, textiles, plastics, cosmetics and pharmaceuticals. However, SNPs has been suggested as reinforcement filler in the polymeric composites in many researches (Ma *et al* 2008, Angellier *et al* 2005)). There are a wide range of polymeric matrixes including both natural and synthetic polymers which have been suggested for the composites with SNPs (Nair and Dufresne 2003).

Food applications of starch nanoparticles

Fat replacers

A specific application of SNPs is the use as fat replacers in foods. Fat replacers are basically the non-fat substances that act like fat in foods. Or in more specific words these are the substances which imitate the organoleptic or physical properties of triglycerides but cannot replace fat on a gram-for-gram basis. These may be lipid, carbohydrate or protein based. Native and modified starches can sometimes also be used to replace fat. The particle size of the starches is an important factor in determining both the fat-like taste and mouthfeel. The SNPs, because of the small size could be a promising candidate for a fat replacer. It is expected that after blending SNPs with other components, the mixture forms a smooth cream-like substance that has properties similar to fat. Additionally, the use of SNPs may result in calorie decrease by replacing high calorie fat with the carbohydrate (Kim *et al* 2014).

Emulsion stabilizer

Use of starch nanoparticles in emulsion stabilization has also been accomplished (Haaj *et al* 2014, Li *et al* 2012). In a recent study, SNPs were suggested to be used as a stabilizer in oil-in-water emulsions. An emulsion prepared with equivalent volume ratio of water and paraffin could be stabilized by adding an aliquot of SNP dispersion. It was claimed that the addition of SNPs more than 0.02 wt% stabilized the emulsion longer than 2 month of storage without coalescence of oil droplets (Li *et al* 2012). However, the emulsion became phase-separated when the SNPs

melted by heating at 80◦C for 2 h. In another study, the polymer dispersion stabilized by SNPs proved to be stable for several months when the SNP content was above 4 wt% relative to the monomer phase. As the SNP loading increased, the particle sizes of the polymer dispersion decreased steadily, confirmed the key role of the SNPs in the stabilization process (Haaj *et al* 2014).

<u>Pathogen Detection</u>

Nanobiotechnology has its important application in killing food pathogens and is an alternative to antibiotics and chemical decontamination in food supply systems. The basic principle used in making nanoparticles as killer for pathogens are researchers attached cell lytic enzymes to food safe starch nanoparticles, and created a coating with the demonstrated ability to selectively kill pathogens and not harm other nutrients. This method is based on surface attachment of specific pathogens to nanoparticles containing lytic enzymes. Researchers found that viruses that infect bacteria, called phages, inject their genetic material into healthy cells. The phage transforms the host cell into a little factory that generates more phages. Near the end of its life cycle, the original phage creates and releases lytic enzymes, which break down and make holes in cell walls of the infected bacteria. These phages move out of these holes and infect other healthy cells. Solanki *et al* 2013 immobilized phage endolysins on both silica and starch nanoparticles, and reductions of 4.0 $\log_{10}$ CFU/g and 1.2 $\log_{10}$ CFU/g *Listeria*, respectively, were recorded on iceberg lettuce. These nanoparticles may also be able to be incorporated onto the surfaces of equipment and into the infrastructure of food processing premises, in order to minimize cross-contamination.

Packaging component

The SNPs have attracted a lot of interest as a material in barrier films for food packaging. Barrier property of the packaging films focuses primarily on oxygen permeability and water vapor transmission. A decrease in water vapor permeability by the incorporation of maize SNPs has been reported. It was documented that addition of 30–40% waxy maize SNPs led to a significant decrease in water vapor permeability of sorbitol plasticized pullulan film (Kristo and Biliaderis 2007). However, in another study, it was reported a decrease in the permeability by 40% for a cassava starch film by reinforcing with 2.5% SNPs (Garcia *et al* 2009). The SNPs may also improve the barrier properties with respect to oxygen permeability. It was also demonstrated that the SNPs from waxy maize starch could reduce the oxygen diffusion and permeability of a nanocomposite film prepared with natural rubber (NR) (Angellier *et al* 2005). This finding was attributed to the platelet-like SNPs, which might have blocked the migration of oxygen molecules through the film.

Conclusion

For the past few decades, the nanoparticles originated from polysaccharides (e.g., starch, cellulose, chitin, etc. have been extensively studied in their preparation and utilization. Despite the great potentials of starch nanoparticles for industrial applications, more research should be done on the efficient nanoparticle production on large industrial scale. Additionally, starch

nanoparticles have a strong tendency to aggregate, so the recovery as powder products became difficult. However, the extensive hydrophilic nature of the SNPs provides the significantly high reactivity, which may be one of the advantages of the starch nanoparticles. Starch nanoparticles can be readily used as an additive in fo industry.

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Table 1: By **Tester, R. F.,Karkalas, J.** (2002) Polysaccharides. II. Polysaccharides from eukaryotes. In: Vandamme EJ, De Baets, S., and Steinbuchel, A. eds. *Starch in biopolymers*, Vol. 6. Weinheim: Wiley-VCH, 381–438.

Characteristics of starch granules from different botanical sources						
Starch	Type	Shape	Distribution	Size (µm)		
Barley	Cereal	Lenticular (A-type)<comma> spherical (B-type)	Bimodal	15-25		
Maize (waxy and normal)	Cereal	Spherical/polyhedral	Unimodal	Feb-30		
Amylomaize	Cereal	Irregular	Unimodal	Feb-30		
Millet	Cereal	Polyhedral	Unimodal	12-Apr		
Oat	Cereal	Polyhedral	Unimodal	3-10 (single)		
				80 (compound)		
Pea	Legume	Rentiform (single)	Unimodal	10-May		
Potato	Tuber	Lenticular	Unimodal	5-100		
Rice	Cereal	Polyhedral	Unimodal	3-8 (single)		
				150 (compound)		
Rye	Cereal	Lenticular (A-type)	Bimodal	Oct-40		
		Spherical (B-type)		10-May		
Sorghum	Cereal	Spherical	Unimodal	20-May		
Tapioca	Root	Spherical/lenticular	Unimodal	May-45		
Triticale	Cereal	Spherical	Unimodal	30-Jan		
Sago	Cereal	Oval	Unimodal	20-40		
Wheat	Cereal	Lenticular (A-type)	Bimodal	15-35		
			Spherical (B-type)	10-Feb		

Table 2: Methods of preparation of starch nanoparticles and their average particle sizes obtained.

Method of preparation of starch nanoparticles	Average Size of diameter obtained (nm)	References
Acid hydrolysis	40-70	Kim *et al* 2012
Emulsion crosslinking	100	Zhou *et al* 2014
Nanoprecipitaion	50-100	Ma *et al* 2008
High pressure homogenization	100-500	Shi *et al* 2011
Ultrasonication	30-100	Haaj *et al* 2013
Reactive Extrusion	160	Song *et al* 2011
Gamma irradiation	20-30	Lamanna *et al* 2013
Enzymatic treatment followed by acid hydrolysis	145	LeCorre *at al* 2011
Combination of hydrolysis and ultrasonication	50-90	Kim *et al* 2013
Enzymatic hydrolysis and recrystallization	20-100	Sun *et al* 2014
Enzymatic treatment	500	Kim *et al* 2008
Complex formation followed by enzymatic hydrolysis	10-20	Kim *et al* 2009
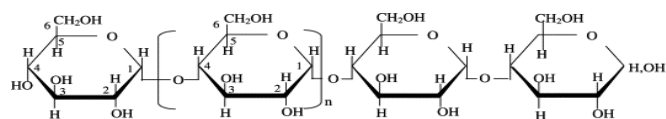
Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).
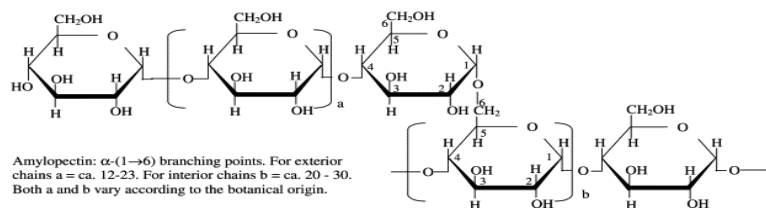
Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 - 30. Both a and b vary according to the botanical origin.

Fig 1: By **Tester, R. F.,Karkalas, J.** (2002) Polysaccharides. II. Polysaccharides from eukaryotes. In: Vandamme EJ, De Baets, S., and Steinbuchel, A. eds. *Starch in biopolymers*, Vol. 6. Weinheim: Wiley-VCH, 381–438.
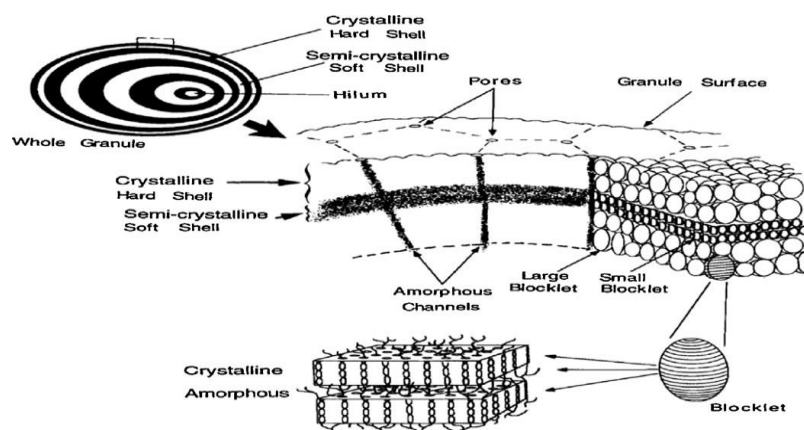
Fig 2: Schematic drawing of hierarchical structure of starch granule (By: Gallant, D.J., Bouchet, B., and Baldwin. P.M.(1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydr. Polym*. **32:** 177.)
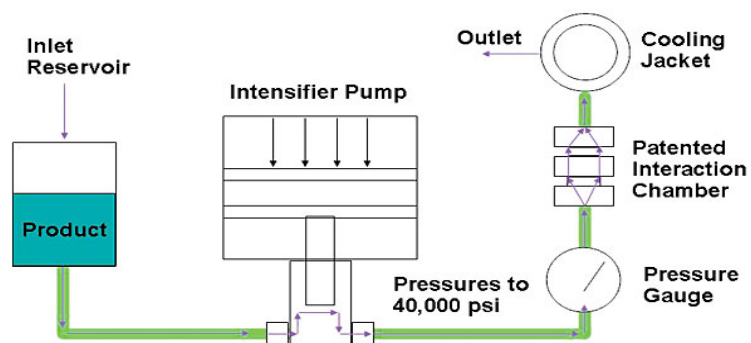
Fig 3:High Pressure Homogenization By Lee, S. Y., Chun, S. J., Kang, I. A., and Park, J. Y. (2009).Preparation of Cellulose nanofibrils by high pressure homogenizer and cellulose based nanocomposite films. *J of industrial engineering and chemistry* **15**:50-55.
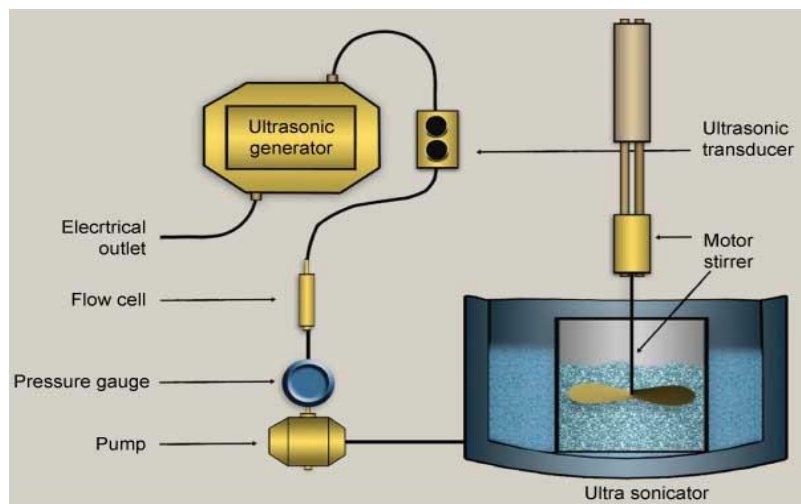
Fig 4: Ultrasonication Process By Kentish, S., Wooster, T. J., Ashokkumar, M., Balachandran, S., Mawson, R., and Simons, L. (2008). The use of ultrasonics for nanoemulsion preparation. *Innov. food sci. & emerg. Technol.* **9:**171 – 2.
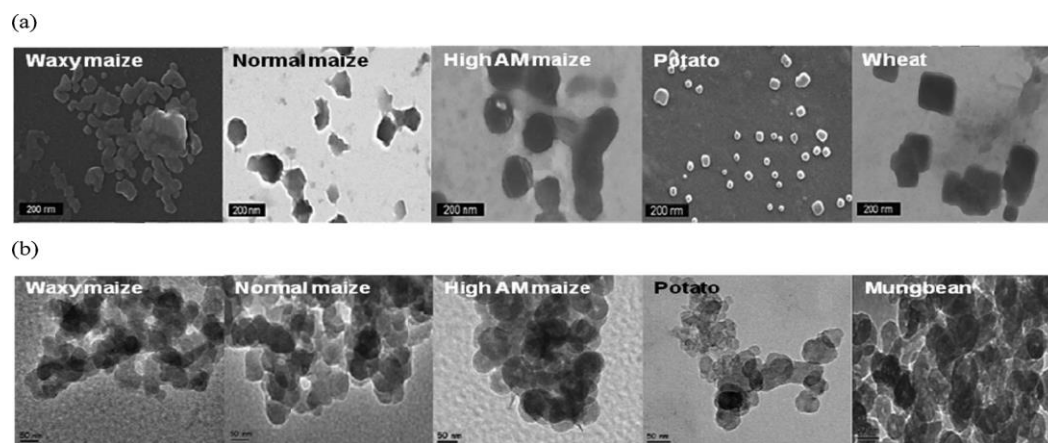
(a)

(b)

Fig 5: Morphology of starch nanoparticles (SNPs) obtained by different botanic origins. (a) SEM micrographs after acid hydrolysis using diluted sulfuric acid (3.16 M, 5 days] (LeCorre *et al* 2011). (b) TEM micrographs after acid hydrolysis using diluted sulfuric acid (3.16 M, 5 days) (Kim *et al* 2012).

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