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(54) Title: MODIFICATION OF STARCHES AND PRODUCTION OF NEW BIOPOLYMERS USING SUBCRITICAL FLUID TECHNOLOGY

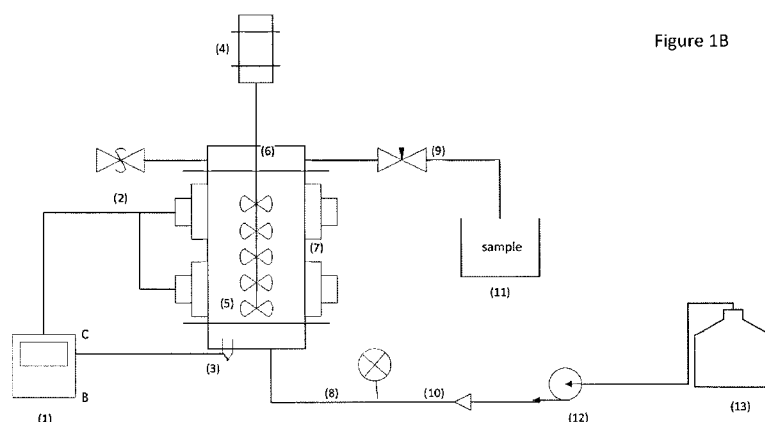


Figure 1B

(57) Abstract: Modified starch, film products, and methods of producing the same using subcritical fluid (sCF) processes are provided. In some embodiments, modified starch can be produced by a process based on combining at least starch, a phenolic compound, a solvent and a buffer and exposing the mixture to a subcritical fluid process. Similarly, in some embodiments, a film product can be produced from at least a starch, a phenolic compound, an emulsifier and a solvent, by subjecting the mixture to a subcritical fluid process and then drying the film. The resulting film product can have antioxidant and/or antimicrobial activities and can have a variety of applications. An apparatus used for the subcritical fluid process is also provided.

**TITLE:       MODIFICATION OF STARCHES AND PRODUCTION OF NEW  
BIOPOLYMERS USING SUBCRITICAL FLUID TECHNOLOGY****TECHNICAL FIELD:**

The present disclosure relates to methods to modify starches and the products resulting therefrom, and more particularly, to methods using subcritical fluids to modify starches from plants to produce films, bioplastics, biofilms and gels resulting therefrom.

**BACKGROUND:**

Current technologies used to form films, or biofilms, are typically petrochemical and protein based. They use large quantities of toxic petrochemical solvents or high amounts of undesirable chemicals. The global reduction of crude oil reserves and its environmental effects have led to the search of cheap, abundant, non-toxic, biodegradable and recyclable raw materials for the production of plastics. These criteria can be fulfilled by agricultural biomass by-products.

Biodegradable polymers, such as bioplastics, are an eco-friendly alternative to traditional plastics. The utilization of biodegradable polymer by-products can contribute to reduced amounts of waste and sustainable production and therefore have been given increasing attention worldwide, mainly in the food packaging industry.

Bioplastics can be obtained from natural polymers, known as biopolymers (for example, starch). The three main types of bioplastics are biopolyethylene, polylactic acid (PLA) and thermoplastic starch. Biopolyethylene production

(approximately 200,000 tonnes per year) requires huge amounts of bioethanol and therefore competes with other industrial sectors, like the fuel and chemical industries, for the use of bioethanol. PLA is produced, at approximately 120,500 tonnes per year, through numerous processing steps (biomass hydrolysis/fermentation/lactic acid purification/polymerization) and is therefore expensive to produce. In contrast, thermoplastic starch, produced at approximately 122,900 tonnes per year, is the least expensive to make as it is produced by mixing a starch and a plasticizer like glycerol (a by-product of the biodiesel industry).

Starch based biodegradable polymers have attracted increasing attention due to its price and high availability worldwide (Li, M., Liu, P., Zou, W., Yu, L., Xie, F., Pu, H., Chen, L. (2011). *J Food Eng*, 106, 95-101, incorporated herein by reference). The consumption of starch and starch derivatives on the EU market reached 8.9 million tons in 2011 with 33% for non-food applications (European starch industry association (2011) <http://www.aaf-eu.org/european-starch-industry>, incorporated herein by reference). Starch, mainly from wheat, potato, rice, and corn are the major sources for these non-food applications, such as cardboard, adhesives, plates, cups, etc. (Halley, P. J., Truss, R. W., Markotsis, M. G., Chaleat, C., Russo, M., Sargent, A. L., Sopade, P. A. (2007). *Ame Chem Soc*, 978, 287-300, and Wittaya, T. (2012). *Structure & Function of Food Engineering*. InTech, incorporated herein by reference).

The main applications for thermoplastic starch are in the pharmaceutical (drug capsules, coating, etc) and packaging (foamed, compostable bags and films) sectors.

The plastic packaging industry in Canada accounts for 34% of total plastics production. This industry is high-growth, innovative and extremely diverse and is largely made up of small and medium-sized enterprises that manufacture both flexible and rigid packaging. The packaging industry in Canada produces approximately \$15 billion, with exports of \$5.5 billion (85% to the United States) and imports total about \$6 billion. Approximately 60% of all packaging is utilized by the food industry.

The packaging industry has produced a new and reliable approach to overcome the problem in the food industry resulting from the application of antimicrobial dips or sprays on the product surface, which could interact with food components with undesired effects. In particular, antimicrobial packaging techniques, such as antimicrobial biofilms, made from bioplastics, can be used to protect meat or fruits. Biofilms using thermoplastic starch have the additional benefit of having oxygen-barrier properties and in some cases can replace aluminum foil to protect oxygen-sensitive foods.

Conventional extrusion methods have been used to produce films from corn and potato starches as well as from potato and wheat flour (for example as described in Fishman, M. L., Coffin, D. R., Onwulata, C. I., & Willett, J. L. (2006). *Carbohydr Polym.* 65: 421-429, Galdeano, M. C., Grossmann, M. V. E., Mali, S.,

Bello-Perez, L. A., Garcia, M. A., & Zamudio-Flores, P. B. (2009). *Materials Sci Eng: C*, 29, 492-498, Thuwall, M., Boldizar, A., & Rigdahl, M. (2006). *Carbohydrate Polymers*, 65, 441-446 and Zhang, B., Dhital, S., Haque, E., & Gidley, M. J. (2012). *Carbohydr Polym.* 90(4), 1587-1594, all incorporated herein by reference). Typically, before the extrusion step, starch, water and plasticizers are well-mixed. This mixture is then forced through the barrel of an extruder where starch is gelatinized and restructured into a free-flowing material. The material emerges from the die and the mixture is cooled (for example see Su, B., Xie, F., Li, M., Corrigan, P. A., Yu, L., Li, X., & Chen, L. (2009). *Intern J Food Eng*, 5, incorporated herein by reference). However, there are challenges with this process, for example the high viscosity and poor process ability of starch-based materials, resulting in low quality films, as described in Mościcki, L., Mitrus, M., Wójtowicz, A., Oniszcuk, T., Rejak, A. & Janssen, L. (2012). *Food Res Intern.* 47(2), 291-299, herein incorporated by reference. Further, in traditional starch modification processes, various chemicals such as sodium hydroxide are used for binding specific chemicals to starch. These residual chemicals need to be removed from the final product at high cost.

Other known processes use technology such as wet chemical reactions and traditional extrusion that require long procedures. These prior technologies demonstrate a number of drawbacks and limitations and are costly.

Bio-polymers have also been used in the pharmaceutical industry to control drug release from tablets and there is a continued need to develop new,

safe and effective bio-polymers for controlled release. Modified starches have been used for various pharmaceutical purposes, such as fillers and matrix formers in capsules and tablet formulations (for example as described in M. K. Kohke, H. R. Chuech and C. J. Rhodes, *Drug Dev. Ind. Pharm.*, 18, 2207. (1992) and K. P. R. Chowdary and P. V. Venkateswara Rao, *Drug Dev. Ind. Pharm.*, 20, 799. (1994), both herein incorporated by reference). One important modification to starch for use in this industry is to produce acetylated starch. For example, starch acetate is reported (for example in M. Tarvainen, R. Sumwen, S. Peltonen, P. Tiihonen and P. Paroneni, *J. Pharm. Sci.*, 91, 282 (2002), and O. Korhonen, P. Raatikainen, P. Harjunen, J. Nakari, E. Suihko, S. Peltonen, M. Vidgren and P. Paronen, *Pharm. Res.*, 17, 1138 (2000), herein incorporated by reference) to have excellent bond forming ability and is suitable for coating and controlled release applications. The known methods to modify starch to produce starch acetate include using aqueous caustic solutions (for example as described in Charles L. Mehlretter, Charles L. Mehlretter, Patent # US3553196, High-Amylose Starch Acetate, 1971 and Dennis Neigel, Lawrence J. Scotchie, Martin M. Tessler, Patent # US5629416, Method of preparing crosslinked starch esters, 1997, herein incorporated by reference) and recently using pure ionic liquids-caustic catalyzers (as described in Reijo Aksela, Vesa Myllymaeki, Kemira Oyj, Patent # WO 2007006848 A1, Starch etherification method, 2007, herein incorporated by reference). The reaction time needed for

these processes using aqueous caustic solutions are from 30 to 90 min and around two hours for using ionic liquids.

There remains a need to provide a method and product that can overcome the short comings of the prior art.

**SUMMARY:**

Modified starch and film products and methods of producing the same using subcritical fluid (sCF) processes are provided. In some embodiments, modified starch can be produced by a process based on combining at least starch, a phenolic compound, a solvent and a buffer and exposing the mixture to a subcritical fluid process. Similarly, in some embodiments, a film product can be produced from at least a starch, a phenolic compound, an emulsifier and a solvent, by subjecting the mixture to a subcritical fluid process and then drying the film. The resulting film product can have antioxidant and/or antimicrobial activities and can have a variety of applications. An apparatus used for the subcritical fluid process is also provided.

A method for producing a modified starch product is provided. The method can include mixing starch with a phenolic compound, a solvent and a buffer to form a mixture, sealing the mixture in a high pressure reactor; and exposing the mixture to a subcritical fluid process where there is sufficient temperature and pressure to cause a chemical reaction resulting in the production of a modified starch product. This chemical reaction can take less than 60 minutes.

In some embodiments the method for producing a modified starch product can also include, mixing a strengthening agent, for example cellulose and lignin, an essential oil, spice and/or enzyme with the mixture prior to sealing in the high pressure reactor.

The method of producing a modified starch can further include drying the modified starch product after the chemical reaction, and/or extracting the starch from a plant source prior to mixing it with at least the phenolic compound, the solvent and the buffer.

In another embodiment, a method for producing a film is provided, the method including combining starch, which can be a modified starch, a phenolic compound, an emulsifier and a solvent to form a mixture, subjecting the mixture to a subcritical fluid process where the temperature is above 100°C and the pressure above 5 bar for a period of time to produce a film solution, and drying the film for example by oven, freeze drying or supercritical carbon dioxide drying.

The method for producing a film may also include comprising combining an additive, for example an essential oil or spice, with the mixture and/or extracting the starch and/or phenolic compound from a plant, crop or crop by-product.

In another embodiment a process for producing a film is provided. This process can include placing a mixture of starch, a phenolic compound and an emulsifier into a high pressure reactor, pumping water into the high pressure reactor, increasing the pressure to at least 5 bar and the temperature to at least

100°C, stirring the mixture and returning the mixture to atmospheric pressure and room temperature.

In another embodiment a film for use in the packaging industry is provided. The film can include at least a starch for providing a modifiable base structure and a phenolic compound for providing anti-oxidant properties. The film can further include cellulose or lignin to provide strength and/or an essential oil for providing anti-microbial activity. The film can be made from crop industry by-products, for example potato, barley, wheat, cassava, beets and oats.

In another embodiment a film having certain physical characteristic is provided. These can include a thickness between 56 and 180  $\mu\text{m}$ , a tensile stress between 0.5 and 92 MPa and an elongation percentage between 0.1% and 91%. The film can also have anti-oxidant activity and/or water activity in the range of 0.1 to 0.4.

In another embodiment the use of the film described above in the food packaging industry for packaging or bagging meat, fruit, and frozen products, in shopping bags, electronics, contact lenses or breath strips and/or in pharmaceutical, cosmetic, biomedical or medical applications is provided.

In another embodiment an apparatus for use in subjecting a mixture to a subcritical fluid process is provided. The apparatus can include a high pressure reactor for receiving a mixture, a solvent reservoir for storing fluid, a liquid delivery pump fluidly connected to the high pressure reactor and solvent reservoir for delivering fluid from the solvent reservoir to the high pressure

reactor, a temperature controller for controlling the temperature of the mixture, a pressure controller for controlling the pressure inside the high pressure reactor, and a stirrer for stirring the mixture.

**BRIEF DESCRIPTION OF THE DRAWINGS:**

Figure 1A depicts an embodiment of a sCF system.

Figure 1B depicts another embodiment of the sCF system.

Figure 2 depicts an embodiment of protocols for comparative evaluation of subcritical fluid (sCF) processes for the production of films from starch rich by-products of potato industry.

Figure 3 depicts an embodiment of a scanning electron microscopy (SEM) analysis of film products.

Figure 4 depicts an embodiment of a SEM analysis of film products where plates a & c are duplicates of plates A & C from Figure 3.

Figure 5 depicts an embodiment of a microscopic analysis of an original starch (a) and its modification/crosslinked with lignin (b).

Figures 6A and 6B depict an embodiment of a film product where, after the sCF process, the film product was treated with ethanol and centrifuged before drying.

Figure 7 depicts an embodiment of a film product where the film product was dried using freeze drying to produce a porous gel.

Figure 8 is a cross section view of the film when dried in a petri dish, as shown in Figures 3 and 4.

Figures 9A and 9B depict an embodiment of a film product as produced without the use of glycerol.

#### DETAILED DESCRIPTION OF EMBODIMENTS:

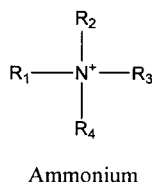
In this disclosure:

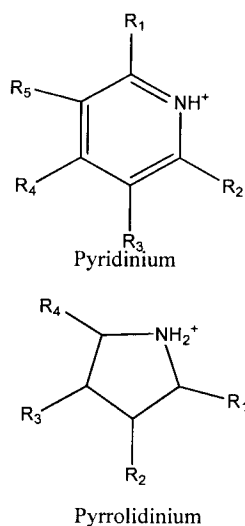
**"biocompounds"** can mean compounds that may be obtained from biomasses, for example, starches, carbohydrates, pectine, and other chemicals;

**"biomass"** can mean natural and biological material that may comprise a high water content such as, for example, plants, crops or crop byproducts, from which biocompounds can be obtained;

**"carbohydrates"** can mean organic biocompounds that consist only of carbon, hydrogen and oxygen, wherein the hydrogen:oxygen atom ratio is typically 2:1 (e.g. starch);

**"ionic liquid"** can mean an ionic salt with a melting temperature below 100°C. Ionic liquids may comprise one or more anionic and one or more cationic component. The present disclosure provides ionic liquids that may be synthesized to improve starch modification, and may be various types of ionic liquids, including, without limitation, ionic liquids having a cationic component having the following formula:

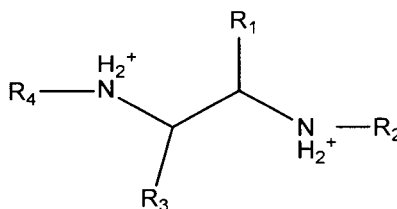




wherein R1-R4 are independently selected from the group consisting of C1-C6 alkyl, alkenyl, hydroxyalkyl, haloalkyl, alkoxyalkyl; C6-C10 aryl, C8-C16 alkylene-aryl or C1-C7 alkyl sulphonic; derivatives thereof, and mixtures thereof.

The present ionic liquid can comprise an anionic ion, which when paired with the cationic ion can form an ionic liquid. The anionic ion can be selected from the group consisting of halides, C1-C6 carboxylates, C1-C6 alkyl sulfates, mono/di-C1-C10 alkyl sulfosuccinates, or C1-C7 alkyl sulphonic; derivatives thereof, and mixtures thereof.

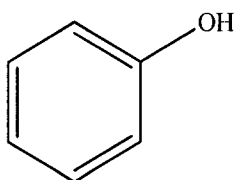
Also, in some experiments, the cationic component of the ionic liquid can have the formula:



wherein R1-R4 are independently selected from the group consisting of C1-C6 alkyl, alkenyl, hydroxyalkyl, haloalkyl, alkoxyalkyl; C6-C10 aryl or C8-C16 alkylenearyl; C1-C6 alkoxyalkyl, hydroxyl or hydrogen. The anionic part has two ions components, such as halides, C1-C6 carboxylates, C1-C6 alkyl sulfates, mono/di-C1-C10 alkyl sulfosuccinates, or C1-C7 alkyl sulphonic; derivatives thereof, and mixtures thereof;

**"ionic liquid solution"** can mean a mixture of one or more ionic liquids and one or more organic or inorganic solvents, such as water and ethanol;

**"polyphenols"** can mean organic biocompounds having large multiples of phenolic structure units including, without limitation:



In some embodiments, natural phenolic compounds, such as gallic acid, chlorogenic acid, caffeic acid or sinapinic acid can be used;

**"subcritical fluid state"** can refer to circumstances where an ionic liquid or ionic liquid solution exhibits a fluid state at the range between the liquid's or solution's boiling point and critical point. For example, common points may be temperatures ranging from 50°C to 500°C and high pressures ranging from 5 bar

to 500 bar. The present disclosure describes the importance of pressure in some embodiments to increase the reaction rate of carbohydrates or starches;

**“Modification of starch”** can refer to the use of the starch from any plant or crop to produce esters or ethers of starch by addition of an aqueous media or non-aqueous media (ionic liquid solution) at different conditions, including its subcritical state. By way of example the use of acetic anhydride and ionic liquid solution at subcritical state to treat the starch produced an esterified product;

**“biopolymer”** can mean polymers produced using biocompounds. Biopolymers contain monomeric units that are covalently bonded to form larger structures. Modified starch produced in some embodiments can be considered a biopolymer; and

**“Film”** can mean the film produced by the use of a biopolymer. In some embodiments, the film can be produced by a subcritical fluid process. The term “film” can be used interchangeably with biofilm and gel.

Methods for producing modified starch and films using a subcritical fluid (sCF) process are provided. In some embodiments this process can create films that can have a variety of applications. In some embodiments the modified starch produced by the sCF process can be used to prepare the film. In some embodiments the starch used in the sCF reaction can be derived from a by-product created by the produce industry.

#### Method for Producing Modified Starch

The method can relate to modifying starch structures through interactions with chemicals, such as phenolics and other additives (e.g. citric acid, phosphates, canola oil, etc) using a sCF process to produce a modified starch that can be used in a variety of applications, including in the production of film (as described below). Starch can be purchased in purified form, or it can be extracted from plants, crops or crop by-products (as described below). These crops may be, for example, potato, corn, oat, cassava, beets or barley.

The starch can be mixed with a phenolic compound, a solvent, for example water or aqueous ionic liquid, and a buffer to produce a mixture. In addition, a strengthening agent, for example cellulose or lignin, can also be added to the mixture. Other modifiers, such as an oil, essential oil or spice, for example canola oil, rosemary essential oil, cloves, cinnamon or other spices, can also be added to the mixture. As well, enzymes, for example lipase can be added to the mixture.

This mixture can then be loaded into a reactor, for example a high pressure reactor, which may also be referred to herein as a vessel, and treated at subcritical condition, namely high temperature, for example at least 60°C, preferably above 100°C, and high pressure, for example above 5 bar, for a period of time sufficient to allow the reaction to produce a modified starch solution. This modified starch solution can further be dried, depending on the desired use or application of the modified starch. For example, in some embodiments, the

modified starch can be precipitated with ethanol and the precipitate can be dried at 40°C.

If used, the ionic liquid can be recycled and reused. If a phenolic compound is used, it can be, in some embodiments, gallic acid, sinapic acid, chlorogenic acid, ferulic acid, caffeic acid or other known phenolic compounds.

It is contemplated that the application of this sCF process can be extended towards the modification of other carbohydrates, polysaccharides, pectines, proteins, fats and lignins as well as synthetic polymers.

Modified starch products that can be produced using the sCF process can include citric starch, acetate starch, phosphate starch, oleic ester starch, and other starches with antioxidant/antimicrobial activities or properties.

Without any limitation to the foregoing, a method of producing modified starch is further described by way of the following examples.

## **Examples**

### Example 1: Modification of Starch I: Esterification of starch using canola oil

Extracted starch from potato with a purity of 86.2% was used in some embodiments of the process to obtain acetate starch in a dried ionic liquid medium. The 2.50 g of starch was modified using a mixture of 25 g of n-methyl-2-hydroxyethylammonium acetate, 0.25 g of Lypase enzyme, 26.1 mg of sinapic acid and 0.2 grams of canola oil. This mixture was loaded into a vessel which was maintained at 60°C and 250 bar with a reaction time of 60 min. After the reaction, the mixture inside the vessel was washed with 10mL of ethanol,

centrifuged (3500 rpm and 10 min) and dried in an oven at 40°C for 48 hr. The modified starch resulted in a clear yellow powder of approximately 2.64 g. This modified starch had a starch content of 80%. Furthermore, the Lipase enzyme was recovered from the ethanolic ionic liquid solution by filtration and the ionic liquid was purified by drying at 40°C for 5 hr.

Example 2: Modification of Starch II: Esterification of starch using citric acid and monosodium phosphate

Extracted starch from potato with a purity of 86.2% was used in some embodiments of the process to obtain citric starch in an aqueous medium. The 4.01 g of starch was modified using a mixture of 40 g of water, 2.2 g of citric acid, 7.1 mg of sinapic acid, and 0.39 g of monosodium phosphate. This mixture was loaded into a vessel which was maintained at 100°C and 100 bar with a reaction time of 30 min. After the reaction, the mixture inside the vessel was washed with 100 mL of ethanol, centrifuged (3500 rpm and 10 min) and dried in an oven at 40°C for 48 hr. The modified starch resulted in a clear white powder of approximately 4.70 g. This modified starch had a starch content of 80%.

Example 3: Modification of Starch III: Starch-lignin cross-linked

Extracted starch from potato with a purity of 86.2% was used in some embodiments of the process to obtain starch cross-linked with lignin in an ionic liquid medium. The 4.66 g of starch was modified using a mixture of 46 g of n-methyl-2-hydroxyethylammonium acetate, and 0.21 g of lignin. This mixture was loaded into a vessel which was maintained at 100°C and 200 bar with a reaction

time of 30 min. After the reaction, the mixture inside the vessel was washed with 100 mL of ethanol, centrifuged (3500 rpm and 10 min) and dried in an oven at 40°C for 48 hr. The modified starch resulted in a yellow powder of approximately 5.20 g. This modified starch had a starch content of 79.6%. The ionic liquid was purified by drying at 40°C for 5 hr. Examples of starches modified using this method can be seen in Figure 5.

#### Method for Producing Film

The present method can comprise dissolving starch, modifiers and polyphenols in subcritical fluids, and consequently obtaining a structure of gelatinized modified starch using a sCF process.

Starch, which can be extracted from plants or crops, such as potato, corn and barley, can be mixed with a phenolic compound and an emulsifier, and then modified under subcritical conditions, using the sCF process described below. The starch may be a modified starch. In some embodiments, the phenolic compound may also be extracted from a plant, crop (for example potato, corn, barley or other cereals) or crop by-product (for example potato peels).

In some embodiments, the solution of starch, a phenolic compound and an emulsifier can be further mixed with additives, for example essential oils, spices, or a strengthening agent, in order to alter the final functional characteristics of the film.

Once the sCF process has been completed, the liquid film product can be dried. This drying can be done in a variety of ways depending on the desired characteristics of the final product (as described below).

### **Starch Extraction**

Starch from potato by-products (culls/starch and peels) can be modified and used for the production of biopolymers. High starch abundance and low starch cost allows for starch to be an excellent candidate for a material to create films. In preliminary studies, carbohydrates (e.g. starch), and phenolic acids of potato peels can be obtained using traditional and subcritical fluid (sCF) technologies (for example see Singh, PP, Saldaña, MDA. (2011) *Food Res. Intern.* 44: 2452-2458 and Alvarez, V.A. & Saldaña, M.D.A. (2012) *Proc. 16th World Congress of Food Sci & Technol*, Brazil, herein incorporated by reference).

Starches, for example, from potato cull and other crop by-products can be extracted following conventional methodologies described in the literature (for example Vasanthan, T., Bergthaller, W., Driedger, D, Yeung, J. & Sporns, P. (1999) *Food Res Intern.* 32(5): 355-365, incorporated herein by reference). The purity of the isolated dried starch can be determined using the Megazyme™ method.

### **Phenolic Extraction**

Phenolic compounds can be extracted using sCF process, for example, from potato peels, at optimized processing conditions (as described in Singh, PP, Saldaña, MDA. (2011) *Food Res. Intern.* 44: 2452-2458 and Alvarez, V.A. &

Saldaña, M.D.A. (2012) Proc. 16th World Congress of Food Sci & Technol, Brazil, herein incorporated by reference). Total phenolics of 30 mg/g potato peel can be obtained (as shown in Alvarez, V.A. & Saldaña, M.D.A. (2012) Proc. 16th World Congress of Food Sci & Technol, Brazil). Furthermore, studies with phenolic acids of potato peels can show antimicrobial effects against *Escherichia coli*, *Bacillus cereus*, and *Salmonella typhimurium* (for example as shown in Sanchez-Maldonado, AF, Schieber, A, Gänzle, MG. (2011) J Appl Microbiol. 111: 1176-1184, herein incorporated by reference). Total phenolic content can be determined as mentioned above.

### **Subcritical Fluid Apparatus and Process**

The sCF apparatus, shown in Figure 1A, can be used to produce films. The apparatus can comprise a high pressure reactor, or vessel, for receiving a mixture, a liquid delivery pump for delivering solvent, for example water, to the high pressure reactor, a stirrer for stirring the mixture in the high pressure reactor, temperature and pressure controllers for controlling the temperature and pressure in the high pressure reactor, a safety valve with a rupture disc for safety in case of overpressure in the system, and on/off valves to allow the solvent to flow to the vessel and the solution to flow to the sample collection. The apparatus can be operated in batch mode, as a semi-continuous system or as a continuous system.

Similarly, the sCF apparatus (1) shown in Figure 1B can be used to produce films. The apparatus can comprise a temperature controller 2, a safety

valve 3, a thermocouple 4, to provide the temperature in the vessel, a motor or stirrer driver 5 that can control the velocity of the stirring (and which can be controlled by a SEPAREX<sup>TM</sup> control panel), a double helix stirrer 6 designed for used with viscous solutions, a valve 7, at least one band heater 8, to provide heat to the apparatus, a pressure gauge 9 which can indicate the pressure of the system, a pressure regulator 10 that can allow a controller to maintain or increase pressure in the system, a one-way valve 11, a sample collection area 12 where the product is obtained, a pump 13, and a solvent reservoir where the solvent(s) are stored.

A solution of starch, a phenolic compound, and an emulsifier, can be placed in the high pressure reactor of the sCF system. The solution can be mixed with a subcritical fluid, for example water, which can be pumped into the high pressure reactor from the solvent reservoir, and then exposed to temperatures above 100°C and pressures of at least 5 bar, while being stirred. Preferably the temperature range is between 100°C and 150°C, more preferably the temperature range is between 120°C and 140°C and more preferably the temperature is 130°C. Preferably the pressure range is between 50 bar and 250 bar.

After this reaction under subcritical conditions has been completed, the resulting homogenized film can be dried, for example by being transferred into a 10 or 20 cm diameter petri dish and dried, for example in an oven at 35°C for 3 days, or shorter time at higher temperature. Alternatively, the homogenized film

may be treated with ethanol and then centrifuged before being dried in an oven. The homogenized film may also be dried using freeze drying or supercritical carbon dioxide drying.

As outlined above, the starting mixture may also contain additional additives in order to achieve specific functional characteristics in the film.

As examples, two different approaches for producing film starting with plant or crop material are outlined in Figure 2.

In approach 1, the starch can be extracted from a crop by-product, known as “culled potato”. Similarly, another crop by-product, the “potato peel” is used to provide the phenolic compound, using a subcritical fluid (sCF) extraction process. The residues from both extraction processes can be discarded. The starch and phenolic compounds can then be used in the sCF process, as described above.

In approach 2, the raw materials (potato culls and peels) can be homogenized and directly processed by the sCF process. The potato culls and peels can be mixed at different proportions, homogenized to produce a slurry and placed in the high pressure reactor of the sCF system to undergo the sCF process, as described above, to produce a film.

The sCF process can rely on unique properties (e.g. variation of fluid polarity at high temperatures under pressure, etc). As such, the sCF process herein described can extract/react compounds of biomass and reduce potato by-product particle size, providing uniform films. In addition, the energy required for

the sCF process can be lower than that of processes to vaporize fluids thereby providing additional benefits to the user.

As well, the short time (from 1 to 30 minutes) and temperature (from 60 to 200°C) of the sCF process can be lower as compared to prior methods, for example the casting method.

Without any limitation to the foregoing, the method of producing films/gels are further described by way of the following examples.

### **Example**

Pure potato starch, or other starches, and a pure phenolic compound can be investigated in the presence and absence of a plasticizer and an emulsifier. Purified starch and a phenolic compound can be mixed in different proportions in combination with emulsifiers and plasticizers to produce films using the sCF process. Different pressure and temperature conditions with constant agitation can be investigated.

Solutions with known amounts of starch, glycerol and water were prepared using approach 1, following the factorial design described in Table 1 below, and placed in the high pressure reactor. The solutions were exposed to the sCF process to produce films, which are shown in Figures 3 and 4. Cross sectional examples of these films, as depicted in Figure 8, show that the films are homogeneous and thin. Other examples of films are shown in Figures 6, 7 and 9, and are described in further detail below. Additional experiments examine ratios

up to 60mg of a phenolic compound to 1g of starch and 1g of glycerol to 1g of starch.

**Table 1.** Factorial design for the subcritical fluid process

Run	Pressure (bar)	Heating time (min)	mg phenolic/ g starch	g starch / g water	g glycerol / g starch	Water (g)	Temperature (°C)
7	5	15	3	0.05	0.5	50	100
4	50	15	3	0.05	0.5	50	100
1	5	30	3	0.05	0.5	50	100
8	50	30	3	0.05	0.5	50	100
6	5	15	10	0.05	0.5	50	100
2	50	15	10	0.05	0.5	50	100
5	5	30	10	0.05	0.5	50	100
3	50	30	10	0.05	0.5	50	100

### Film Characteristics

In some embodiments, the film produced by the methods or processes described above may have antimicrobial and/or antioxidant activity due to the use of a phenolic compound in its production. The use of a strengthening agent provides improved mechanical properties, such as increased tensile stress and modulus.

The film may also have improved tensile strength and elongation over the prior films due to the addition of various additives, described earlier, and have a stability of more than 60 days due to the anti-oxidant and anti-microbial components of the additives used.

In some embodiments, the film having certain physical characteristic. For example, these can include a thickness between 50 and 200 $\mu$ m, more preferably between 56 and 180 $\mu$ m, a tensile stress between, 0.5 and 100 MPa, more preferably between 0.5 and 92 MPa and an elongation percentage between 0.1% and 95%, more preferably between 0.1% and 91%. The water activity of the film can be between 0.1 and 0.6, more preferably between 0.1 and 0.4. The film can also have anti-oxidant activity and/or anti-microbial activity.

Physico-chemical and mechanical characterization can be performed on the films to determine their specific characteristics.

## **Experiments**

Films obtained by the above methods and processes can be stored in a humidity control chamber (30 $\pm$ 2% RH) at 25°C until characterized for composition, mechanical, physicochemical and functional properties as described below. All characterizations can be performed at least in duplicates.

Film composition can be determined for total carbohydrates (starch, fiber and sugar contents) and phenolics using abovementioned methodologies described earlier (Singh, PP, Saldaña, MDA. (2011) Food Res. Intern. 44: 2452-2458; Alvarez, V.A., Cahyadi, J, Xu, D., Saldaña, MDA. (2014) Journal of Supercritical Fluids. 90: 8-17, herein incorporated by reference).

Physicochemical properties can be determined by the following methodologies, for example:

a) Water vapor permeability: The gravimetric modified cup method based on ASTM E96-92 (described in McHugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993) J Food Sci, 58, 899–903, herein incorporated by reference) can be used to determine water vapor permeability.

b) Water solubility: Solubility of the films in water can be measured following the method described in Gontard, N., Guilbert, S., & Cuq, J. L. (1993). J Food Sci, 58, 206–211 (herein incorporated by reference), with slight modifications. Two hundred milligram of the film can be placed into 20 mL water at 25°C using a water bath. A 50 µL of supernatant can be obtained at four different sampling times between 0 and 24 h, and then can be stored at -20°C until analysis.

c) Color: A colorimeter can be used to measure L, a, and b values by CIELAB coordinates. The colorimeter can be calibrated using white and black standard tiles.

d) Water activity: The tested film strips can be cut into 200 mm × 200 mm pieces using a small blade. Water activity of the films can be determined at 25°C using an AquaLab™ serie 4TE water activity meter.

Mechanical properties can be measured by the following methodologies, for example:

a) Mechanical properties: The American Society of Testing and Materials (ASTM) standard method D 882-01 (as described in ASTM. (1997) D822-01. Philadelphia, PA: American Society for Testing and Materials, herein

incorporated by reference) can be used to measure tensile properties of tensile strength (TS), elastic modulus (EM), percent elongation at break (%E) of films, and film thickness with an Instron series 5967 (30kN) tension tester fitted with a 30 kg load cell. The initial grip separation can be set at 30 mm and the crosshead speed can be set at 4 mm/min. Crosshead speed previously optimized for the other starch films can be used. The tested film strips can be cut into 100 mm × 10 mm pieces using a small blade.

Functional properties can be measured by the following methodologies, for example:

a) Radical-scavenging activity: The solutions obtained from the solubility analysis can be analyzed for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity according to the method previously used (as described in Sarkar, S., Alvarez, V.H. & Saldaña, MD.A. (2012) *Proc. 10<sup>th</sup> International Symp. on Supercritical Fluids*, San Francisco, USA, herein incorporated by reference).

b) Antimicrobial activity: the different films antimicrobial activity can be determined following established literature protocols.

Film stability: The film stability over storage time (1-60 days) can be evaluated at room (25°C) and refrigeration/frozen (4°C and -18°C) temperatures by the same characterization methodologies described above.

Without any limitation to the foregoing, certain characteristics of various films is further described by way of the following examples.

## Examples

Examples of some of the films that can be produced are shown in Figures 6, 7 and 9.

The films depicted in Figure 6 were exposed to ethanol and then centrifuged after the sCF process was completed but before the precipitate was dried. The shape of these films suggest they may be useful in the biomedical industry (for example for implantation) or in the pharmaceutical industry (for example for use as a capsule).

The gels depicted in Figure 7, were created by freeze drying the solution obtained after the sCF process. This gel is very porous and flexible and therefore may be useful in the pharmaceutical industry as a substrate which can be impregnated with drugs. A similar result can be obtained where supercritical carbon dioxide is used to dry the solution.

The films shown in Figure 9 were made without the inclusion of glycerol.

Table 2 shows the mechanical results of the films produced using sCF after combining potato starch and the phenolic compound gallic acid.

**Table 2.** Mechanical results for subcritical experiments with potato starch and gallic acid.

Sample #	Thickness ( $\mu\text{m}$ )	Elongation (%)	Tensile stress (MPa)	Modulus (MPa)
1	150.18	22.82	20.01	1122.92
2	144.70	35.57	16.87	938.05
3	142.70	23.96	17.21	914.86
4	144.48	25.52	18.23	1009.26
5	133.30	24.25	18.57	865.94
6	133.48	41.15	9.01	402.31
7	146.73	18.92	19.31	1083.83
8	152.08	27.81	15.94	914.38

For comparison, Table 3 shows the mechanical results done to films produced by other methods.

**Table 3.** Mechanical test results for other experiments and the literature

Sample #	Thickness ( $\mu\text{m}$ )	Elongation (%)	Tensile stress (MPa)	Modulus (MPa)
Conventional method (S1)	180.65	17.38	9.41	386.42
Conventional method (without gallic acid) (S2)	207.50	40.91	8.75	115.92
EF31*	92.05	10.78	21.03	1182.23
EF32*	84.95	34.52	3.65	94.20
EF33*	83.20	4.46	30.04	1723.49
<b>Extrusion (from literature)</b>				
Waxy corn starch <sup>a</sup>	230	$2.28 \pm 0.38$	$12.68 \pm 2.41$	$730.7 \pm 120.0$
Regular corn starch <sup>a</sup>	230	$4.23 \pm 0.35$	$15.66 \pm 2.14$	$866.6 \pm 124.9$
High amylose corn starch <sup>a</sup>	230	$4.95 \pm 0.80$	$23.82 \pm 1.85$	$1217.2 \pm 334.7$
Potato starch and potato flour <sup>b</sup>	---	4-11	0.8-2.6	24-36
Corn starch and stearic acid <sup>c</sup>	----	45.79–90.83	0.23–2.91	2.89–37.94
PCL+starch+glycerol	70	40	20	---
Polycaprolactone <sup>d</sup>	1037	>340	22	---
Polycaprolactone <sup>d</sup>	51	37	102	---

\*EF31, EF32 and EF33: potato peel subcritical water extracts. <sup>a</sup>Li et al. (2011),

<sup>b</sup>Zhang et al. (2012), <sup>c</sup>Pushpadass et al. (2009), <sup>d</sup>Myllymaki et al. (1998).

Table 4 shows the gallic acid release and anti-oxidant activity of the films.

**Table 4.** Gallic acid release and antioxidant activity of the film (in water at 25°C and 24 h)

Sample #	Releasing (%)	Inhibition of DPPH
1	17.17	$47.65 \pm 1.72$
2	58.44	$77.30 \pm 8.37$
3	65.95	$78.88 \pm 10.39$
4	22.27	$54.75 \pm 2.65$
5	78.00	$80.38 \pm 11.74$
6	76.72	$78.41 \pm 9.91$
7	51.98	$53.98 \pm 1.95$
8	25.32	$45.10 \pm 10.95$

S1	25	73.7 $\pm$ 1.1
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A physicochemical property, specifically the water activity of the film, after 3 days is shown in Table 5.

**Table 5.** Water activity for films obtained after 3 days.

Sample #	Water activity
1	0.0874
2	0.0998
3	0.0947
4	0.0997
5	0.1078
6	0.1146
7	0.1048
8	0.0880
EF31	0.1446
EF32	0.1622
EF33	0.1561
S1	0.1305
S2	0.1304

#### Applications

A number of traditional potential applications exist for the present films, for example, packaging and preservation of foods susceptible to oxidation like foods rich in omega-3, packaging of fresh produce and bakery products, and use in skin care applications, like cosmetic care products. Other applications for films with antimicrobial activity include the packaging of meats, fruits and frozen products. It is further contemplated that the present films can be used in electronics and associated applications, contact lenses and breath strips.

The scope of the claims should not be limited by the embodiments as set forth in the examples herein, but should be given the broadest interpretation consistent with the description as a whole.

Although a few embodiments have been shown and described, it will be appreciated by those skilled in the art that various changes and modifications can be made to the embodiments described herein. The terms and expressions used in the above description have been used herein as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding equivalents of the features shown and described or portions thereof, it being recognized that the invention is defined and limited only by the claims that follow.

The teachings provided herein can be applied to other methods, not necessarily the method described herein. The elements and acts of the various embodiments described above can be combined to provide further embodiments.

These and other changes can be made to the invention in light of the above description. While the above description details certain embodiments of the invention and describes certain embodiments, no matter how detailed the above appears in text, the invention can be practiced in many ways. Details of the method may vary considerably in their implementation details, while still being encompassed by the invention disclosed herein.

Particular terminology used when describing certain features or aspects of the invention should not be taken to imply that the terminology is being redefined

herein to be restricted to any specific characteristics, features, or aspects of the invention with which that terminology is associated. In general, the terms used in the following claims should not be construed to limit the invention to the specific embodiments disclosed in the specification. Accordingly, the actual scope of the invention encompasses not only the disclosed embodiments, but also all equivalent ways of practicing or implementing the invention.

The above description of the embodiments of the invention is not intended to be exhaustive or to limit the invention to the precise form disclosed above or to the particular field of usage mentioned in this disclosure. While specific embodiments of, and examples for, the invention are described above for illustrative purposes, various equivalent modifications are possible within the scope of the invention, as those skilled in the relevant art will recognize. The elements and acts of the various embodiments described above can be combined to provide further embodiments.

While certain aspects of the invention are presented below in certain claim forms, the inventor contemplates the various aspects of the invention in any number of claim forms. Accordingly, the inventor reserves the right to add additional claims after filing the application to pursue such additional claim forms for other aspects of the invention.

## REFERENCES

The following additional references are hereby incorporated into this application by reference in their entirety.

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**WE CLAIM:**

1. A method for producing a modified starch product, comprising,  
mixing starch with a phenolic compound, a solvent and a buffer to form a mixture;  
sealing the mixture in a high pressure reactor; and  
exposing the mixture to a subcritical fluid process where there is sufficient temperature and pressure to cause a chemical reaction resulting in the production of a modified starch product.
2. The method of claim 1, further comprising, mixing a strengthening agent with the mixture prior to sealing in the high pressure reactor.
3. The method of either one of claims 1 or 2, further comprising, mixing an essential oil or spice with the mixture prior to sealing in the high pressure reactor.
4. The method of any one of claims 1 to 3, further comprising, mixing an enzyme with the mixture prior to sealing in the high pressure reactor.
5. The method of any one of claims 1 to 4, further comprising, drying the modified starch product.
6. The method of any one of claims 1 to 5, further comprising extracting the starch from a plant source prior to mixing it with the phenolic compound, the solvent and the buffer.
7. The method of any one of claims 1 to 6, wherein the phenolic compound provides the modified starch with anti-oxidant or anti-microbial properties.

8. The method of any one of claims 1 to 7, wherein the solvent is selected from the group consisting of water, ethanol and an aqueous ionic liquid.
9. The method of any one of claims 2 to 8, wherein the strengthening agent is selected from the group consisting of cellulose and lignin.
10. The method of any one of claims 2 to 7, wherein the solvent is water and the strengthening agent is cellulose and lignin.
11. The method of any one of claims 2 to 7, wherein the solvent is an aqueous ionic liquid and the strengthening agent is lignin.
12. The method of any one of claims 1 to 11, wherein the chemical reaction takes less than 60 minutes.
13. A method for producing a film, the method comprising,  
combining starch, a phenolic compound, an emulsifier and a solvent to form a mixture;  
subjecting the mixture to a subcritical fluid process where the temperature is above 100°C and the pressure above 5 bar for a period of time to produce a film solution; and  
drying the film.
14. The method of claim 13, further comprising combining an additive with the mixture.
15. The method of claim 14, wherein the additive is an essential oil or spice.
16. The method of any one of claims 13 to 15, wherein the starch is a modified starch.

17. The method of any one of claims 13 to 16, wherein the film is dried by oven, freeze drying or supercritical carbon dioxide drying.
18. The method of any one of claims 13 to 17, further comprising extracting the starch from a plant.
19. The method of any one of claims 13 to 18, further comprising extracting the phenolic compound from a plant.
20. The method of either claims 18 or 19, wherein the plant is a crop.
21. The method of claim 20, wherein the plant is at least part of a crop.
22. The method of claim 21, wherein the at least part of a crop is a crop by-product.
23. The method of any one of claims 13 to 22, wherein the subcritical fluid process takes less than 15 minutes.
24. A process for producing a film, comprising,  
placing a mixture of starch, a phenolic compound and an emulsifier into a high pressure reactor;  
pumping water into the high pressure reactor;  
increasing the pressure to at least 5 bar and the temperature to at least 100°C;  
stirring the mixture; and  
returning the mixture to atmospheric pressure and room temperature.
25. The process of claim 24, wherein the process is performed as a batch system.

26. The process of claim 24, wherein the process is performed as a continuous system.
27. A film produced by any one of methods 13 to 23.
28. A film for use in the packaging industry, the film comprising,  
a starch for providing a modifiable base structure; and  
a phenolic compound for providing anti-oxidant properties.
29. The film according to either one of claims 27 or 28 wherein the film is made from crop industry by-products.
30. The film according to claim 29 wherein the crop industry by-products is selected from the group consisting of potato, barley, wheat, cassava, beets and oats.
31. The film of any one of claims 28 to 30, further comprising cellulose or lignin for providing strength to the film.
32. The film of any one of claims 28 to 31, further comprising an essential oil for providing anti-microbial activity.
33. A film having,  
a thickness between 56 and 180 $\mu$ m;  
a tensile stress between 0.5 and 92MPa; and  
an elongation percentage between 0.1 and 91%.
34. The film of claim 33, further having anti-oxidant activity.
35. The film of either of claims 33 or 34, further having water activity in the range of 0.1 to 0.4.

36. The use of the film of any one of claims 27 to 35 in the food packaging industry for packaging or bagging meat, fruit, and frozen products.
37. The use of the film of one any of claims 27 to 35 in shopping bags, electronics, contact lenses or breath strips.
38. The use of the film of any one of claims 27 to 35 in pharmaceutical, cosmetic, biomedical or medical applications.
39. An apparatus for use in subjecting a mixture to a subcritical fluid process, the apparatus comprising,
- a high pressure reactor for receiving a mixture;
  - a solvent reservoir for storing fluid;
  - a liquid delivery pump fluidly connected to the high pressure reactor and solvent reservoir for delivering fluid from the solvent reservoir to the high pressure reactor;
  - a temperature controller in communication with the high pressure reactor for controlling the temperature of the mixture;
  - a pressure controller in communication with the high pressure reactor for controlling the pressure inside the high pressure reactor; and
  - a stirrer proximate the high pressure reactor for stirring the mixture.

1/9

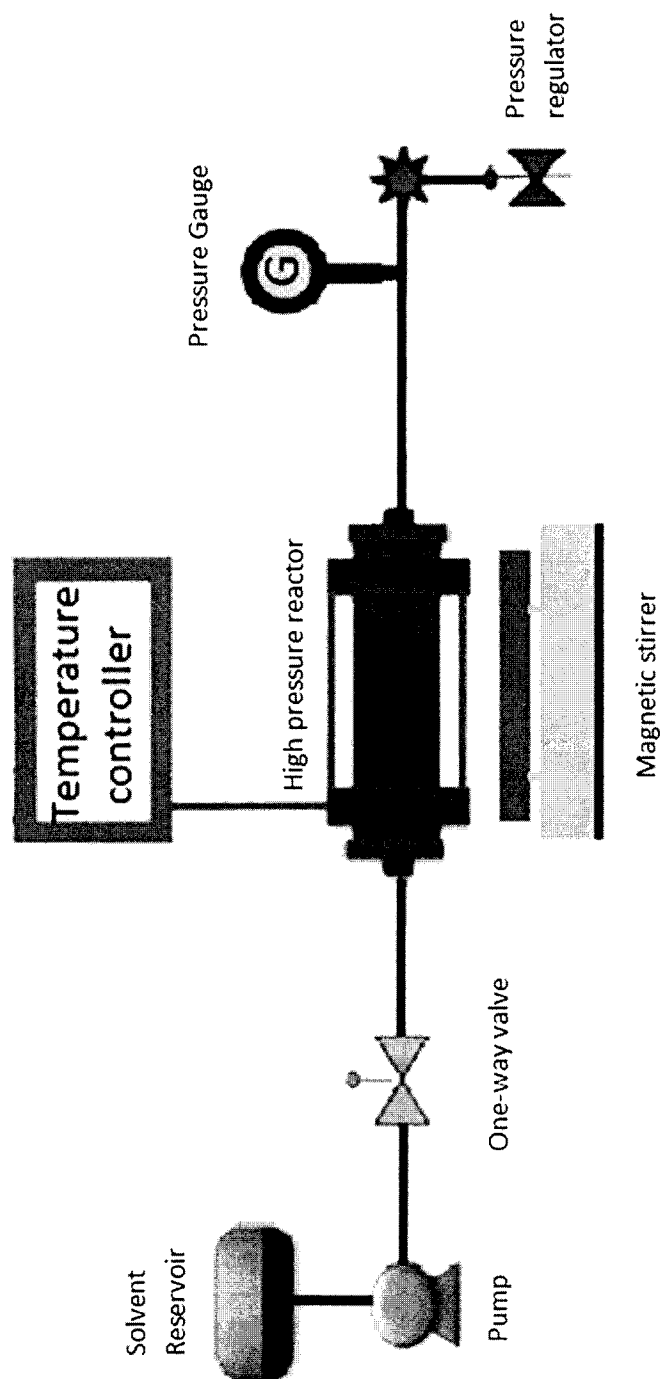


Figure 1A

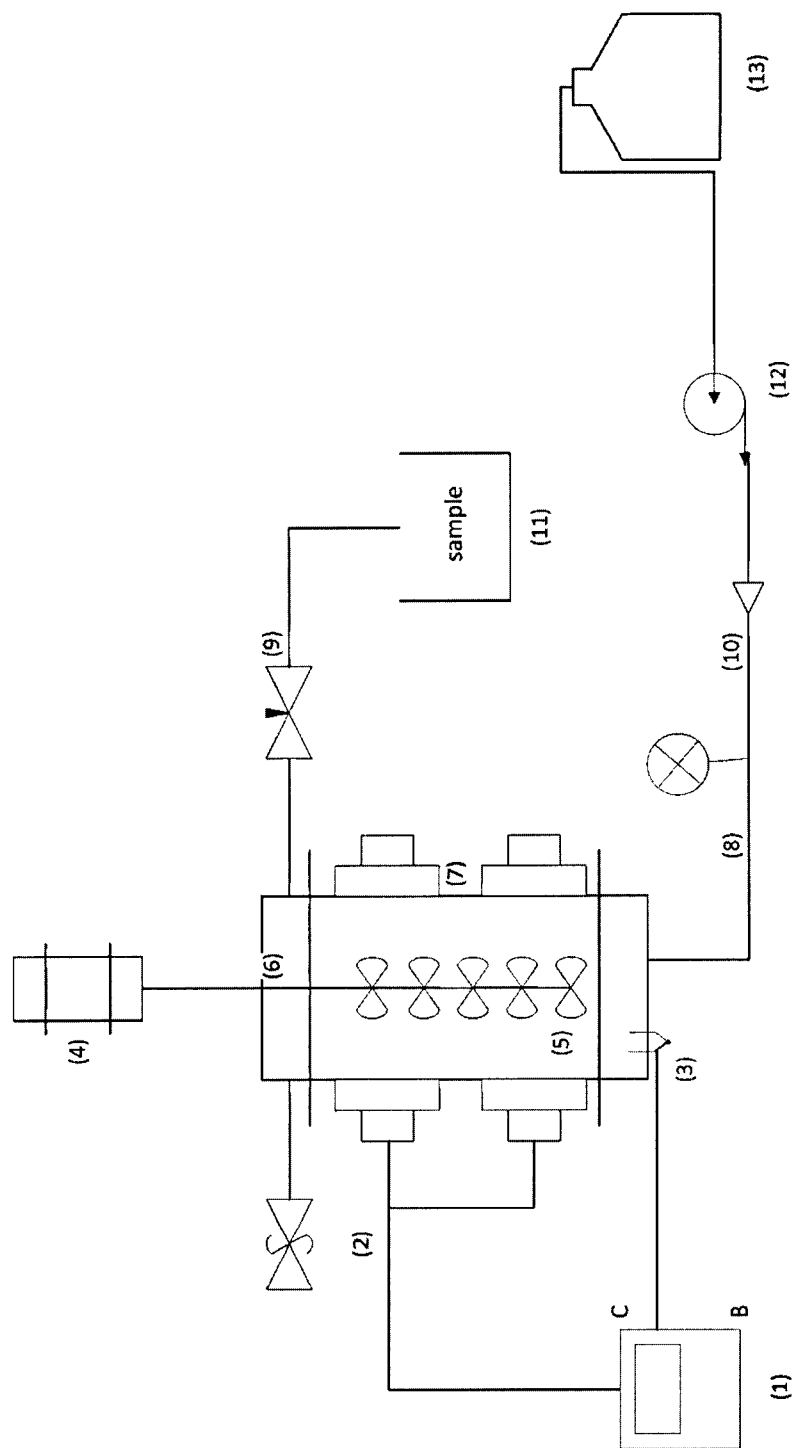


Figure 1B

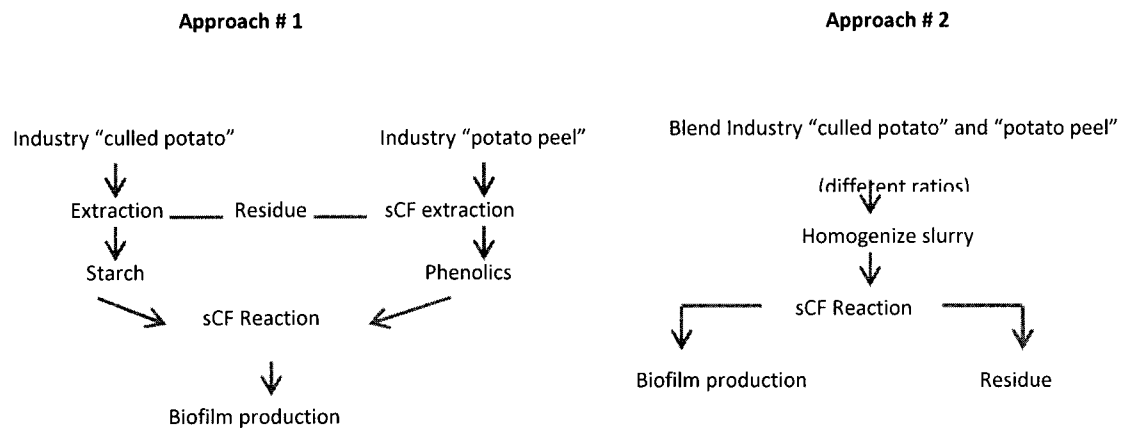


Figure 2

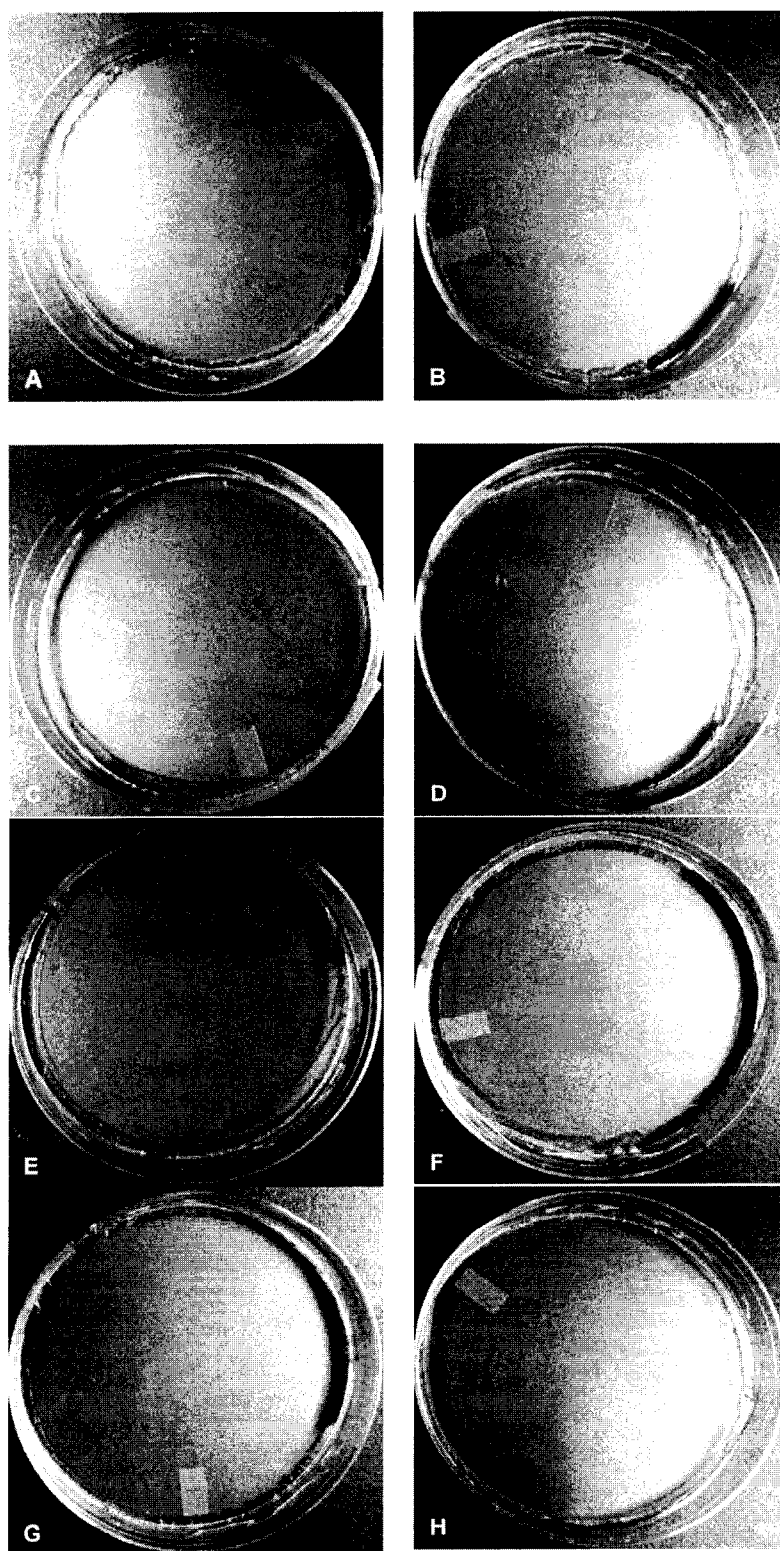


Figure 3

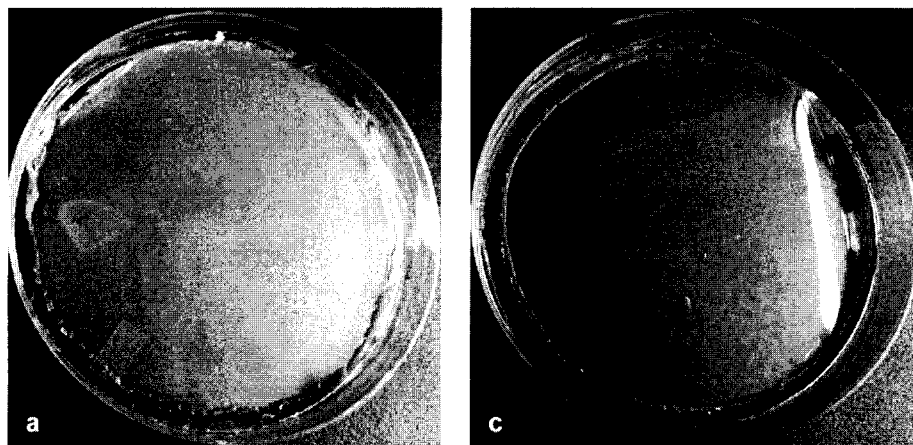
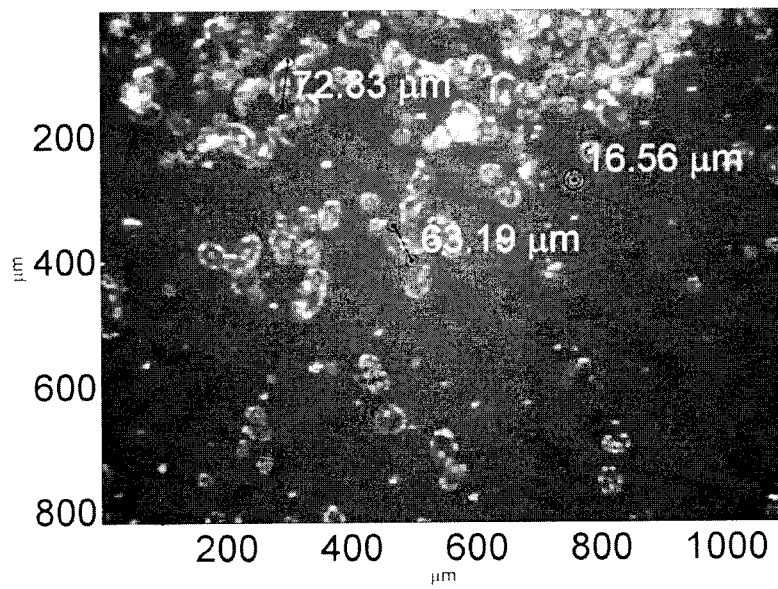
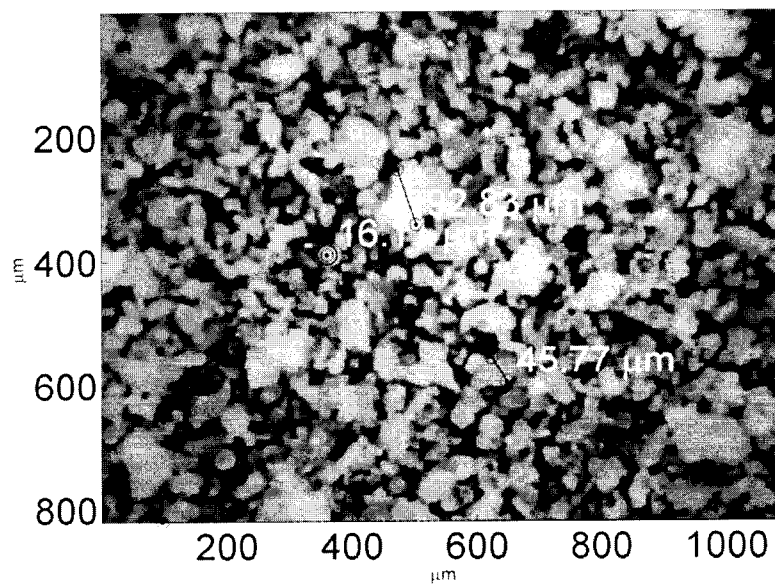


Figure 4



(a)



(b)

Figure 5



Figure 6A



Figure 6B

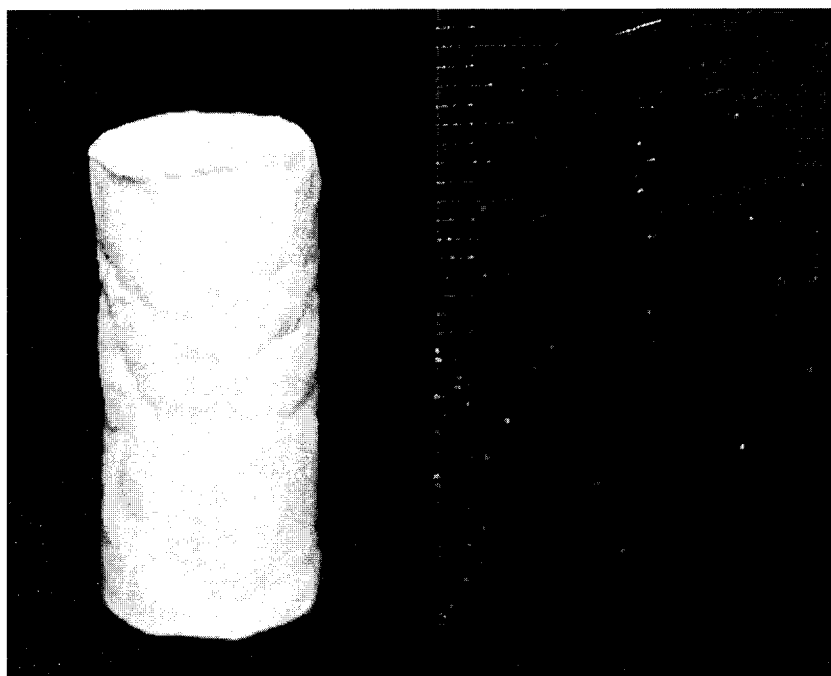


Figure 7

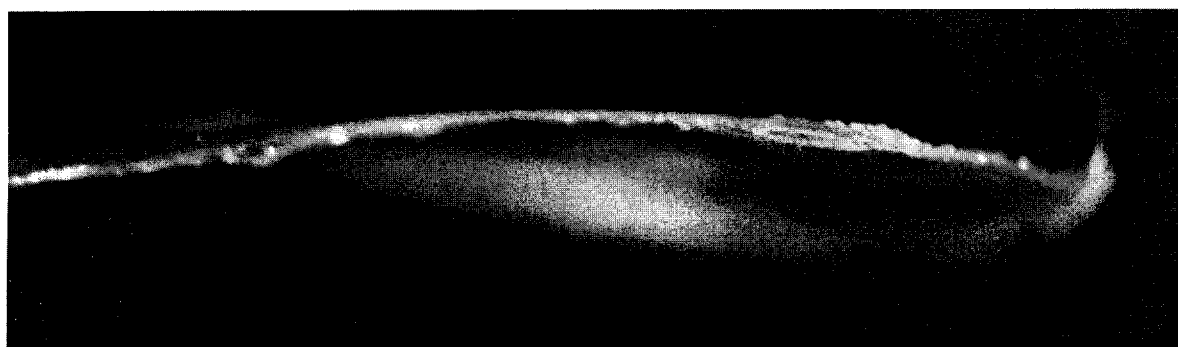


Figure 8

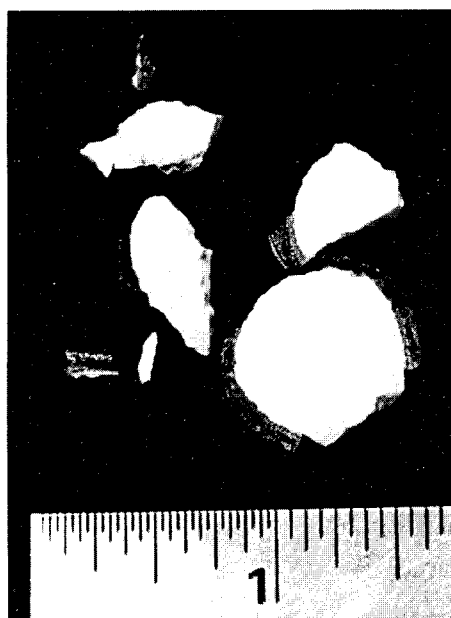


Figure 9A



Figure 9B

## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2014/000432**

## A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C08L 3/04** (2006.01), **B01J 3/00** (2006.01), **C08J 5/18** (2006.01), **C08B 31/00** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**C08L 3/04** (2006.01), **B01J 3/00** (2006.01), **C08J 5/18** (2006.01), **C08B 31/00** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)


Questel Orbit, Canadian Patent Database

Keywords: subcritical, phenol\*, starch, ionic liquid

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 7875262 (KENAR et al.) 25 January 2011 (25-01-2011) Abs, col 3, 156-col 4, 14.	1, 5, 7, 12
A	CA 2796542 (MARAKAINEN, et al.) 27 October 2011 (27-10-2011) Pp 4, 6.	1, 2, 5-8, 12
A	CA 2593411 (WITHERS) 20 July 2006 (20-07-2006) Page 3, line 24, page 6, lines 8-11.	1, 5, 7, 8, 12
A	CA 2610404 (DALY et al.) 7 December 2006 (07-12-2006) Page 13, lines 3-30.	1, 2, 5-12

 Further documents are listed in the continuation of Box C.

 See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search  
21 July 2014 (21-07-2014)Date of mailing of the international search report  
23 July 2014 (23-07-2014)Name and mailing address of the ISA/CA  
Canadian Intellectual Property Office  
Place du Portage I, C114 - 1st Floor, Box PCT  
50 Victoria Street  
Gatineau, Quebec K1A 0C9  
Facsimile No.: 001-819-953-2476

Authorized officer

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## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2014/000432****Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.: 1-12

Claims 1-12 are directed to a method of producing a modified starch product comprising mixing starch with a phenolic compound, a solvent and a buffer to form a mixture, sealing the mixture in a high pressure reactor and exposing the mixture to a subcritical fluid process where there is sufficient temperature and pressure to cause a chemical reaction resulting in the production of a modified starch product.

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III

The claims are directed to a plurality of inventive concepts as follows:

Group A – Claims 1-12 are directed to a method of producing a modified starch product comprising mixing starch with a phenolic compound, a solvent and a buffer to form a mixture, sealing the mixture in a high pressure reactor and exposing the mixture to a subcritical fluid process where there is sufficient temperature and pressure to cause a chemical reaction resulting in the production of a modified starch product.

Group B – Claims 13-32 are directed to a method for producing a film comprising combining starch, a phenolic compound, an emulsifier and a solvent to form a mixture, subjecting the mixture to a subcritical fluid process where the temperature is above 100 degrees centigrade and the pressure above 5 bar for a period of time to produce a film solution and drying the film, and to a film for use in the packaging industries.

Group C – Claims 33-38 are directed to a film having a thickness of 56-180 microns, a tensile strength of 0.5-92 MPa and an elongation percentage of 0.1-91%.

Group D – Claim 39 is directed to an apparatus for use in subjecting a mixture to a subcritical fluid process comprising a high pressure reactor, a solvent reservoir, a liquid delivery pump, a temperature controller, a pressure controller and a stirrer.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/CA2014/000432**

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