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ning of each regular issue of the PCT Gazette.

(54) Title: "EDIBLE FILMS AND COATINGS BASED ON FRUCTOOLIGOSACCHARIDES WITH PROBIOTIC PROPER-
TIES"

(57) Abstract: The present invention relates to "Edible films and coatings based on fructooligosaccharides with probiotic proper-
ties". The product in particular composed of fructooligosaccharides obtained by using fructosyltransferase (FTase) enzyme solution
obtained by fermentation using microbes, a gelling agent like china grass, a plasticiser like glycerol or any other polyol and obtained
by making a solution of these in the required proportion and casting into thin films. The film is edible as all the constituents used
are edible and within permissible limits as prescribed by PFA. The, solution as such can be used as coatings. The method comprises
mixing a film forming agent and at least one stabilizing agent to provide a film-forming mixture; dissolving water-soluble ingredients
in water to provide an aqueous solution; combining the film-forming mixture and the aqueous solution provide a uniform emulsified
gel; casting the uniform gel on a substrate; and drying the cast gel to obtain a film.

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**“EDIBLE FILMS AND COATINGS BASED ON FRUCTOOLIGOSACCHARIDES
WITH PROBIOTIC PROPERTIES”**

The present invention relates to the field of Foods and nutrition. The present invention particularly relates to preparation of edible films or coatings with prebiotic effect. The present invention more particularly relates to preparation of fructooligosaccharide based edible films or coatings with prebiotic effect.

Background of invention:

Ordinary food packages use metal, glass, plastic, foil and wax board containers for protection against external contamination, the effects of atmospheric oxygen and moisture, and for protection against mechanical damage. The food is removed or separated from the package at the time of use. In contrast, when package, in the form of film or coating, as an integral part of the food and consumed as such, the package is classified as soluble or edible films. The most familiar example of edible packaging is sausage meat in casing that is not removed for cooking and eating.

The primary purpose of packaging in general is to retard undesirable migration of moisture, grease or oil, and gaseous components (oxygen, CO₂, volatile flavors), prevent the food from microbial invasion, mechanical damage and breakage, and isolate reactive ingredients. Additional benefits from edible packaging materials as opposed to ordinary packaging materials are that they are edible, their cost is generally low, their use could reduce waste because, they are part of integrated foods, and biodegradable, they can enhance the organoleptic, mechanical, and nutritional properties of foods, they are suitable for wrapping of small pieces or portions of food.

The benefits of edible films indicated above together with environmental legislation, expanding distribution channels, consumer expectations for a variety of fresh foods, need for extended shelf life foods, opportunities for new foods with edible barriers have stimulated the interest in edible packaging.

Some of the applications of edible packaging (films or coatings) are:

Fresh produce: coating - retard moisture transmission, provide protection from

mechanical damage, naturally regulate oxygen and carbon dioxide condition to control respiration.

5 Dried fruits coating and wrapping - maintain desirable moisture content, prevent stickiness and clumping.

Meat and fish wrapping and coating - prevent oxidation, moisture loss and contamination, maintain freshness.

10 This invention relates to dissolving orally consumable films. The films are used to deliver probiotic health ingredients like fructooligosaccharides. The films can also be used to deliver pharmaceutically active agent, flavour and other active food ingredient.

15 Fructooligosaccharides (FOS) is functional food ingredient that has the potential to improve the flavor and physicochemical characteristics of food. They possess properties beneficial to human health, including non-cariogenicity, low calorific value and the ability to stimulate the growth of beneficial bacteria in the colon.

20 The plasticiser required in this invention are primary, external plasticiser, such as sugars and low molecular weight polyols. The properties of the composition optionally can be further enhanced by using internal and/or secondary external plasticiser. A suitable secondary external plasticiser is water.

25 No reports are available on fructooligosaccharide based edible films. However, there are reports on the preparation of edible films based on proteins, carbohydrates etc

Reference may be made to a process described by Fang et al., (Journal of Food Science, 2002, 67(1), 188-193), wherein a process for the preparation of edible films
30 based on Whey Proteins (WP) is explained. The composition consisting of WP and glycerol were emulsified using a microfluidizer at an input pressure of 3 bars. The films were cast on the plate and heated at 90°C for 20 minutes, before drying the film.

The drawback of the above process is that the emulsification needs an additional step and the additional heating step required before drying.

Reference may be made to a process described by Kim et al., (Journal of Food
5 Science, 2002, 67(1), 218-222), wherein a process for the preparation of edible films based on Highly carboxymethylated starch (HCMS) is described. The composition consisting of HCMS, sorbitol, manitol, xylitol and glycerol were mixed. The composition was heated at 60°C for 5 minutes, before casting and drying the film.

10 The drawback of the above process is that the composition is based on HCMS wherein the CMS used is from 0.48 to 0.6 %, whereas the PFA limit is less than 0.5 %. Also, the additional heating step required before drying.

Reference may be made to a process described by Lee et al., (Lebensm-W-
15 Technologie, 2003, 36, 323-329), wherein a process for the preparation of edible films based on carrageenan and Whey Protein Concentrate (WPC) is described. The composition consisting of carrageenan, PEG, and glycerol were mixed. Another composition consisting of WPC, PEG, CMC, CaCl₂ and glycerol were mixed. The compositions were heated to 70° to 80°C for 30 - 40 minutes, before casting and
20 drying the film.

The drawback of the above process is that the solutions are heated before drying. This is an additional step.

25 Reference may be made to EDIBLE COATINGS AND FILMS TO IMPROVE FOOD QUALITY edited by John M. Krochta et al, (1994), Technomic Pub Comp Inc., New Holland Avenue, Lancaster, Pennsylvania USA, ISBN:1566761131, wherein Edible Coatings and Films Based on Hydrcolloids (Proteins and carbohydrates), Lipids and Resins (Waxes, Shellac, Sucrose fatty acid esters and glycerides) and
30 Composites (Polysaccharides) have been described in detail.

However, the prior art mentioned above are different from the present invention as they are not based on fructooligosaccharides and does not provide prebiotic effect and

other advantages of FOS (explained earlier). Also, films based on protein are hydropihillic by nature, and this limits their application in food packaging. Also, in case of heat induced gelation, wherein most of the prior art is based on film forming solutions have generally been heat treated and then poured onto a hydrophobic surface to set and dry. The disadvantage of this practice is that the film forming solution must be kept under nongelling of low protein concentration and low ionic strength during heat treatment. To eliminate this problem, a combined process of heat treatment and the setting surface for gelation is used. Hence, it is disadvantageous to have a heating step, jut before pouring (Fang et al., Journal of Food Science, 2002, 67(1), 188-193).

The main object of the present invention is to provide a process for the preparation of edible coatings and films. The present invention provides a method for the preparation of edible coatings and films properties and an additional health benefit of prebiotic and different from the prior art as detailed above, without the step of heating.

Accordingly, the present invention provides a process for the preparation of edible films and coatings based on FOS with prebiotic effect, which is different from the prior, which comprises

- a. Preparation of FOS from the culture *Aspergillus oryzae* MTCC 5154 as described in earlier patents using sucrose/jaggery as the substrate (439/DEL/2001; 411/DEL/2001; 87/DEL/2003).
- b. Mixing 12 to 25 % FOS, 0.5 to 1 % CMC, 6 to 12 % of Glycerol in water to make a homogeneous solution.
- c. Agar/China grass (0.5 to 1.5 %) is added and gelatinized by boiling, to improve the film forming properties.
- d. The above solution is used as such for coatings by dipping the product for a period of 3-5 minutes.
- e. The above solution is cast on a plate to form films and the films are dried for a period of 20 min to 24 hrs.
- f. The required properties like tensile strength, water vapour pressure (WVP), opacity and colour are determined.

In an embodiment of the present invention, any plasticisers other than glycerol like polyols, xylitol, manitol etc may be used.

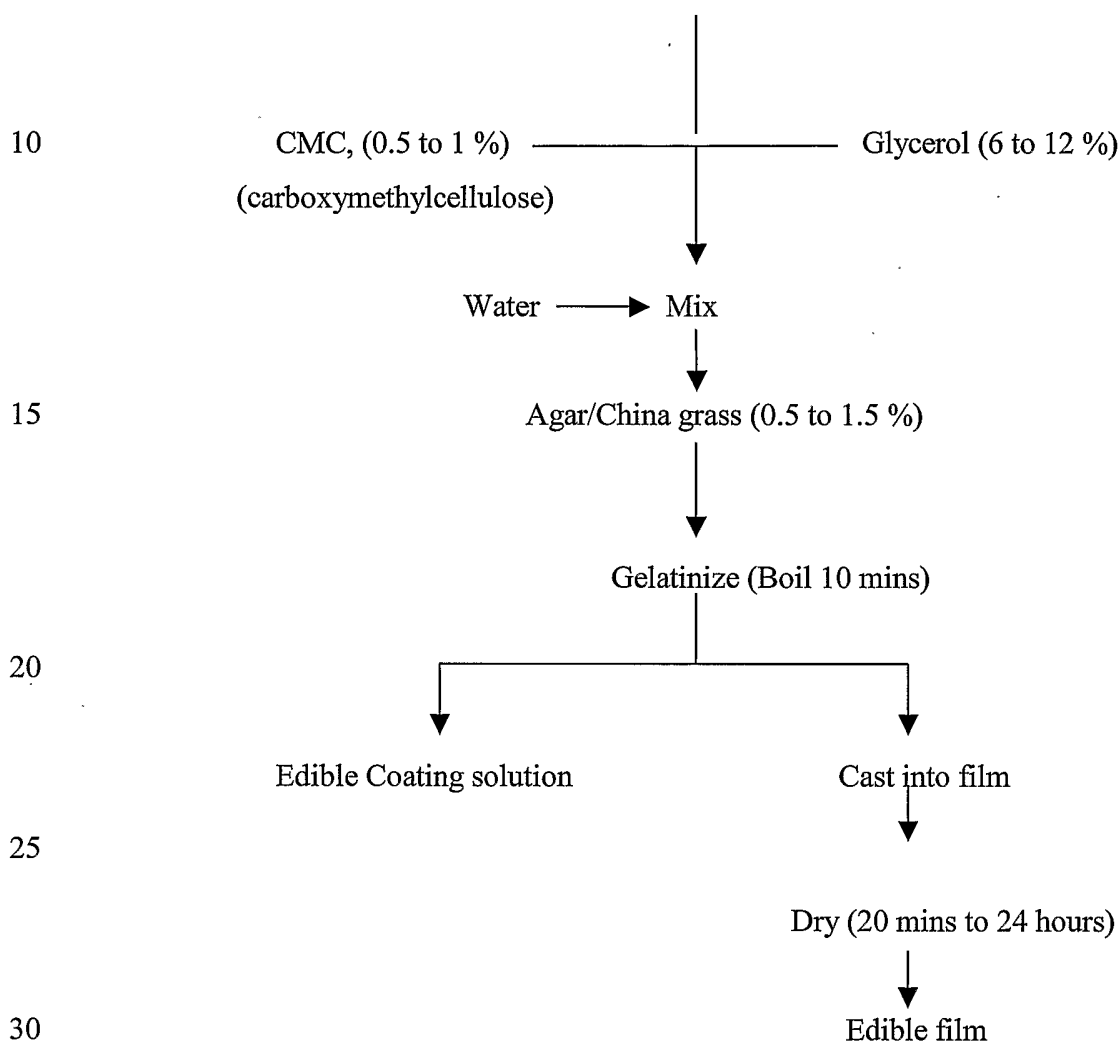
5 In an embodiment of the present invention, the solution may be added with permitted food additives like antimicrobial agents, flavours, colour, vitamins, micro & macronutrients to improve the organoleptic and nutritional properties of the film and the coatings.

10 In another embodiment of the present invention, the film may be dried using conventional oven or infrared dryer for rapid drying.

In another embodiment of the present invention, the films may be cast on a glass plate, or on a continuous belt to obtain suitable film dimensions depending on the application.

15

A process for the preparation of edible coatings and films based on FOS with prebiotic effect is illustrated in the following flow charts.

Flow chart 1:**5 Fructooligosaccharide (FOS) form Fructosyl transferase (FTase) (12 to 25 %)**

The **novelty** of the process is that the edible films and coatings have been prepared using FOS as the base with suitable permitted additives like plasticiser and gelling agent. The preparation of edible films and coatings based on polysaccharides like starch and proteins like soy/whey isolates are well known. It is for the first time that an fructooligosaccharides (FOS) has been used for preparation of the edible film and coatings. The benefits of FOS like prebiotic effect, anticariogenity and their specific physiological effect on humans especially growth stimulation of beneficial

bifidobacteria in the digestive tract, relief of constipation, decrease total cholesterol and lipid in serum are additional benefits into the film and coatings.

Fructooligosaccharides (FOS) preparation:

5 Fructooligosaccharide (FOS) is prepared in a known method as disclosed in our patent application number 439/DEL/2001. In the said process the fructooligosaccharide (FOS) is prepared as follows:-

1. Process for preparation of fructooligosaccharides, comprises the steps of:

- 10 (a) growing the culture in a medium consisting of 1 % sucrose and 0.2 % yeast extract at pH ranging between 5-6 , at a temperature ranging between 25 – 35 °C at about 250 rpm for 24– 120 hrs to develop the inoculum,
- (b) transferring 5-15 % of the inoculum to a medium consisting of 20 % sucrose, 0.5 % yeast extract, 0.05 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 % NaNO_3 , 0.25 % K_2HPO_4 , 0.25
- 15 % KH_2PO_4 , 0.25 % NaCl and 0.5 % NH_4Cl and growing for a period of ranging between 24 - 120 hrs under submerged fermentation conditions,
- (c) harvesting the entire culture broth,
- (d) separating the pellets from culture fluid by filtration using Whatman 2 filter paper number,
- 20 (e) Incubating the culture fluid with the substrate for 1-36 h at 50-60 °C, pH 5 – 6, and
- (f) analyzing the reaction mixture for the content of FOS, which yielded up to 55 % of the initial sucrose concentration.

25 The following examples are given by way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

EXAMPLE – 1

5 FOS films were prepared by mixing 12% FOS, 1.5% china grass and 0.5% carboxymethylcellulose with 6% glycerol in an aqueous medium. The homogenous solution was cast into film on 3" diameter petriplate and dried in an oven at 60°C for 15-18h. The mechanical and physicochemical properties of the film were determined. The colour value was $L = 21.73, a = -0.70, b = 0.77$ and the tensile strength was 0.59 N, 10 opacity was 0.176 (OD at 660nm). The percent elongation was 44 mm. The film was also tested for microbiological safety and was found to be within limits of safety. The thickness of the film was 0.5 – 0.54 mm. The water vapour transmission rate (WVTR) with water inside and 0 % RH outside was determined to be 48.54 (h/h m²) and the WVP was calculated to be 9.60 (g mm/kPa h m²).

EXAMPLE – 2

FOS films were prepared by mixing 12% FOS 1.5% china grass and 0.5% carboxymethylcellulose with 6% glycerol in an aqueous medium. The homogenous 20 solution was cast into film on 3" diameter petriplate and dried in an infrared drier at 110°C for 3-4h. The mechanical and physicochemical properties of the film were determined. The colour value was $L = 20.23, a = -0.68, b = 0.75$ and the tensile strength was 0.55 N, opacity was 0.16 (OD at 660nm). The percent elongation was 40 mm. The thickness of the film was 0.55 – 0.59 mm. The water vapour transmission rate 25 (WVTR) with water inside and 0 % RH outside was determined to be 44.25 (h/h m²) and the water vapour pressure (WVP) was calculated to be 9.59 (g mm/kPa h m²).

EXAMPLE – 3

30 FOS films were prepared by mixing 25% FOS, 3 % carboxymethylcellulose with 0.3 % glycerol in an aqueous medium. The homogenous solution was cast into film on 3" diameter petriplate and dried in an oven at 60°C for 15-18h. The mechanical and physicochemical properties of the film were determined. The colour value was $L =$

21.73, a = -0.70, b = 0.77 and the tensile strength was 0.57 N, opacity was 0.175 (OD at 660 nm). The percent elongation was 42 mm. The thickness of the film was 0.22 – 0.25 mm. The water vapour transmission rate (WVTR) with water inside and 0 % RH outside was determined to be 10.12 (h/h m²) and the water vapour pressure (WVP) was calculated to be 3.04 (g mm/kPa h m²).

EXAMPLE – 4

Edible coatings based on FOS were prepared by mixing 12% FOS, 1.5% china grass and 0.5% carboxymethylcellulose with 6% glycerol in an aqueous medium.

A summary of examples indicating the different combinations used are given in Table 1

Table 1: Summary of examples

Example	Film/ Coating	Composition (%)				Drying method
		FOS	CMC	Glycerol	Agar / china grass	
1	Film	12	0.5	6	1.5	Oven
2	Film	12	0.5	6	1.5	Infrared
3	Film	25	0.3	3	0	Oven
4	Coating	12	0.5	6	1.5	---

The characteristics of film are given in Table 2

Table 2 Characteristics of edible film

Characteristics	
Appearance	Translucent, light brown
Tensile strength (N)	0.55 – 0.59
Opacity (OD ₆₆₀)	0.16-0.176
Elongation (%)	40-44 mm
Thickness (mm)	0.23 – 0.57
WVTR (h/h m ²)	34 – 48
WVP (g mm/kPa h m ²)	3 - 10

Medium sized tomatoes were dipped and wrapped using the above products and stored at room temperature (25 – 28°C) and the shelf life is indicated in Table 3. The physical loss of weight of 12 g was taken as the benchmark to identify the shelf life.

5

Table 3: Shelf life of tomato with different storage conditions (No. of days)

Control	Film Coating	Film Wrapped
16	20	31

ADVANTAGES:

- 10 1. It is for the first time that an fructooligosaccharides (FOS) has been used for preparation of the edible film and coatings.
2. Eible films and coatings based on fructooligosaccharides (FOS) have the benefits of FOS like prebiotic effect.
3. Eible films and coatings based on fructooligosaccharides (FOS) have the
- 15 benefits of FOS like anticariogenity effect.
4. Eible films and coatings based on fructooligosaccharides (FOS) have the benefits of FOS like their specific physiological effect on human digestive track especially growth & stimulation of beneficial bifidobacteria in the digestive tract, relief of constipation effect.
- 20 5. Edible films and coatings based on fructooligosaccharides (FOS) have the benefits of FOS like their specific physiological effect on humans like decrease total cholesterol and lipid in blood serum.

25

CLAIMS

1. A process for the preparation of fructooligosaccharide (FOS) based edible films and coatings with prebiotic effect, which comprises the steps of:
 - a). preparing the fructooligosaccharide (FOS) by culturing *Aspergillus oryzae* MTCC 5154 in a known process using sucrose/jaggery as the substrate
 - b). mixing fructooligosaccharide (FOS). at the concentration in the range of 12 to 25 %, carboxymethylcellulose (CMC) at the concentration in the range of 0.5 to 1 % and Glycerol at the concentration in the range of 6 to 12 % in water to obtain a homogeneous solution.
 - c). adding gethayagents such as Agar or China grass at the concentration in the range of 0.5 to 1.5 %,
 - d). boiling the solution of step (c) to gelatinize in order to impart it with improved film-forming properties,
 - e). dipping of food item/product such as tomato in the solution obtained in step (c) for a period of 3-5 minutes in order to coat it with the said solution, OR
 - f). casting, as an alternative to step (e), of the solution obtained in step (c) on a substratum such as plate in order to form films followed by drying for a period in the range of 20 minutes to 24 hrs
 - g). testing the film obtained in step (f) for required properties such as appearance, tensile strength (N), Opacity (OD₆₆₀), percent elongation, thickness, water vapour transmission rate (WVTR h/h m²) and WVP(water vapour pressure).
2. A process as claimed in '1, wherein any plasticisers used are selected from the group consisting of glycerol, polyols, xylitol, manitol etc.
3. A process as claimed in claims 1 and 2, wherein the solution obtained in step (b) of claim 1 is optionally added with permitted food additives.
4. A process as claimed in claims 1-3, wherein food additives added are selected from the group consisting of antimicrobial agents, flavours, colour, vitamins,

micro and macro nutrients in order to improve the organoleptic and nutritional properties of the film and the coatings.

- 5 5. A process as claimed in claims 1-4, wherein the films obtained in the step (f) of claim 1 have the following characteristic features such as translucent light brown appearance, tensile strength (N) in the range of 0.55 - 0.9, Opacity (OD₆₆₀) in the range of 0.16-0.176, percent elongation in the range of 40-44, thickness in the range of 0.23-0.57mm, water vapour transmission rate (WVTR) in the range of 34-48 h/h m² and water vapour pressure (WVP) in the range of 3-10 g mm/kPa h m²
- 10 6. A process as claimed in claims 1-5, wherein food article used is any food article selected from the group consisting of baked or fresh/raw foods of animal or plant origin.
- 15 7. A process as claimed in claims 1-6, wherein food article used is any food article selected from the group comprising of baked food, dairy product, fruits etc.
- 20 8. A process as claimed in claims 1-7, wherein coated tomato fruit with the film obtained in step (g) of claim 1 has the following characteristic features such as shelf life in the range of translucent light brown appearance, tensile strength (N) in the range of 0.55 - 0.9, Opacity (OD₆₆₀) in the range of 0.16-0.176, percent elongation in the range of 40-44, thickness in the range of 0.23-0.57mm, water vapour transmission rate (WVTR) in the range of 34-48 h/h m² and water vapour pressure (WVP) in the range of 3-10 g mm/kPa h m².
- 25 9. A process as claimed in claims 1-8, wherein drying operation carried out in step (f) of claim 1 is performed using conventional oven or infrared dryer for rapid drying.
- 30 10. A process as claimed in claims 1-9, wherein casting of the films carried out in the step (f) of the claim 1 is performed on substratum such as a glass plate or on a continuous belt to obtain suitable film dimensions depending on the application.

11. A process for the preparation of fructooligosaccharide (FOS) based edible films and coatings with prebiotic effect, as explained herein with reference to the examples above.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2006/000082

A. CLASSIFICATION OF SUBJECT MATTER
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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)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 425 961 A (YATKA ET AL) 20 June 1995 (1995-06-20) column 6, paragraph 3 - column 7, paragraph 3; claims; example 134	1
A	WO 03/063620 A (NEDERLANDSE ORGANISATIE VOOR TOEGEPAST- NATUURWETENSCHAPPELIJK ONDERZO) 7 August 2003 (2003-08-07) page 7, paragraph 2; claims	1
A	US 2004/091537 A1 (MILLER GUY W) 13 May 2004 (2004-05-13) paragraph [0015]; claims; table 1	1
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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