The invention relates to a method of extracting galactomannans from fenugreek seeds comprising the steps of: preparing a solution of one or more salts in water, said salts being present in an amount of 0.5 - 10% by weight of the solution, adjusting the pH in the solution to be in the range of 1 to 5 with an acid, keeping the temperature of the solution in the range of 10 - 60 °C, immersing the fenugreek seeds in the solution for between 2 to 72 hours, recovering the galactomannans from the solution and recovering the fenugreek seeds for further processing.
EXTRACTION OF THE HYDROCOLLOIDS FROM FENUGREEK SEED (TRIGNONELLA FOENUM GRAECUM)

FIELD OF THE INVENTION
5 The present invention is concerned with the extraction of valuable substances from plant seeds. In particular, this application deals with a novel and economic process for the extraction of the hydrocolloids from fenugreek seed.

TECHNICAL BACKGROUND
10 The fenugreek plant belongs to the leguminous family and is an annual plant. The plant is native to Western Asia and has since spread widely over Asia, Europe, North Africa and the Middle East. Fenugreek has been cultivated since ancient times and has many uses in traditional medicine.

15 The hydrocolloids in fenugreek seeds are highly branched galactomannans having a galactose:mannose-ratio of about 1:1, in rare cases about 1:2. The galactomannans present in the mucilage of fenugreek seeds may be commercially useful for example in cosmetics (EP 0 742 007), as a dietary fibre supplement (WO 2009/057125) and in pharmaceutical compositions (WO 2009/053652).

20 In WO 2009/057125, extracts from fenugreek seeds are used to provide a dietary supplement. In this case, the hydrocolloids are extracted together with proteins from crushed seeds using various solvents and/or aqueous solutions.

25 A problem associated with many processes used in the prior art to extract galactomannans from fenugreek seeds is that the processes involve extraction with organic solvents and special equipment, in some cases requiring elevated temperatures (EP 0775451, US 2001/024665).

30 In most cases, the seeds are grounded, crushed, milled or otherwise pulverised prior to extraction in order to increase the rate of extraction.

35 A problem associated with the prior art galactomannan extraction processes disclosed or mentioned in the documents cited above is a lack of selectivity whereby
other substances than galactomannans are extracted simultaneously, resulting in
discoloured, bitter tasting and smelling galactomannan products and cumbersome
and costly purification of the galactomannans may be necessary.
It is an object of the present invention to provide an improved method for extracting
galactomannans from fenugreek seeds.

SUMMARY
The invention relates to a method of extracting galactomannans from fenugreek
seeds comprising the steps of:
preparing a solution of one or more salts in water,
said salts being present in an amount of 0.5 - 10% by weight of the solution,
adjusting the pH in the solution to be in the range of 1 to 5 with an acid,
keeping the temperature of the solution in the range of 10 - 60 °C,
immersing the fenugreek seeds in the solution for between 2 to 72 hours,
recovering the galactomannans from the solution and
recovering the fenugreek seeds for further processing.

It has surprisingly been found that a high quality galactomannan product can be
obtained from fenugreek seeds in an extraction process using a salt solution at acidic
pH as extraction medium at comparatively low temperatures in a simple and cheap
process that does not require specialized equipment.

The addition of salt to the extraction medium has several surprising effects.
The galactomannans are released at low temperatures even from whole seeds at an
adequate rate. Enzymatic processes normally being triggered by the immersion of the
seeds in water are at least partly inhibited by the added salts, whereby e.g. the
formation of bitter-tasting substances is diminished and a more neutrally tasting
product may be obtained.

Discolouration of the galactomannan product by brown colour (probably released
from the husk surrounding the seeds) and/or yellow colour (probably released from
the cotyledon) may be drastically diminished due to the use of an acidic solution of salts as extraction medium according to embodiments of the present invention. With the combination of acidic pH and salt in the solution, the extraction process may be run at comparatively low temperature, thereby selectively extracting the gum from the fenugreek seeds, whereby a substantially pure galactomannan product may be obtained.

In embodiments of the present invention the salts are selected from the group consisting of sodium salts, potassium salts, calcium salts barium salts, copper salts, magnesium salts, iron salts and any combination thereof.

It is particularly advantageous to use non-toxic salts in the extraction medium. Although the salts are more or less removed when washing the galactomannan product, it may be desirable to use salts that when present in small amounts are unproblematic when using the galactomannans in applications related to food, cosmetics or pharmaceuticals.

According to preferred embodiments of the invention, NaCl, KCl and/or CaCl₂ are used as salts in the extraction medium.

In further embodiments of the invention the acid is selected from the group consisting of acetic acid, hydrochloric acid, nitric acid and sulphuric acid. Adjusting the pH to obtain an acidic extraction medium is mandatory according to the present invention. Since the beneficial effects of the acid, e.g. the diminishing of product discolouration, is not dependent on the actual acid used, the acid should be cheap and, preferably, not comprise toxic or otherwise harmful substances. A preferred acid, according to an embodiment of the present invention, is acetic acid.

In another embodiment of the invention the step of recovering the galactomannans comprises the steps of depositing the galactomannans with a polar alcohol, re-dissolving the galactomannans in water at least once,
repeating said depositing of the galactomannans and drying the galactomannans.
The steps may be regarded as purifying steps, by which the galactomannans are washed so that salts and other residues from the extraction process are removed. The salts from the extraction solution and washing solutions may be recovered and re-used.

In further embodiments of the invention the pH is in the range of 2 - 4, preferably in the range of 2.5 - 3.5.

It has surprisingly been found by the present inventor that when adjusting the pH to be acidic during the extraction process, a superior galactomannan product is obtained. The obtained galactomannan powder is substantially free of discolouration and undesirable taste, making it useful in applications, where the purity of the galactomannans is important. Such applications may among others comprise food additives, cosmetic products and/or medicinal products.

In another embodiment of the invention the temperature of the solution is in the range of 20 - 50 °C, preferably in the range of 30 - 40 °C.

The extraction process according to the present invention may be run at comparatively low temperatures due to the composition of the extraction solution. This reduces costs and is gentle towards other valuable components of the seeds which may be recovered upon further processing of the seed mass after extraction from the galactomannans. Also, the galactomannans themselves are prone to hydrolysis and other unwanted chemical reactions that may reduce the quality of the galactomannan product.

In an embodiment of the invention the extraction method comprises the step of infusing the fenugreek seeds with a solution of one or more salts prior to the step of immersing the fenugreek seeds in the acidic salt-containing solution.

When infused with a solution of salts, the fenugreek seeds will swell, but no galactomannans are released from the seeds. Infusion with a solution of salts prior to
extraction may speed up the extraction process, whereby the time required at elevated temperatures may be diminished, and thereby further protecting temperature sensitive substances in the fenugreek seeds.

Preferred salts for the infusion-solution are selected from the group consisting of sodium salts, potassium salts, calcium salts barium salts, copper salts, magnesium salts, iron salts and any combination thereof. In particular, NaCl, KCl and/or CaCl₂ may be used according to embodiments of the present invention.

According to a preferred embodiment of the present invention the fenugreek seeds are used as whole seeds in the extraction.

A particular advantage of this embodiment of the present invention is that galactomannans in high yield are selectively being extracted from whole fenugreek seeds, leaving the seeds more or less intact after extraction. The seeds contain valuable oils and other substances that are still comprised in the solid seeds after the gum is extracted when using whole seeds in the extraction process. Further processes to obtain e.g. valuable oils may therefore advantageously be performed on the seed mass after galactomannan extraction.

According to another embodiment of the present invention the fenugreek seeds are dry milled prior to extraction.

Dry milling crushes the fenugreek seeds, whereby a faster rate of extraction may be obtained due to the larger surface area of the milled seeds when compared to whole seeds.

In another embodiment of the present invention the fenugreek seeds are wet milled prior to extraction.

Wet milling crushes the fenugreek seeds, whereby a faster rate of extraction may be obtained due to the larger surface area of the milled seeds when compared to whole seeds. The use of a wetting medium may prevents the formation of dust during the milling process.
In further embodiments of the invention, the wetting medium is a solution of one or more salts.
The use of a solution of one or more salts as wetting medium during the wet milling process provides for the beneficial effect of minimizing unwanted enzymatic activity in the wetting medium during the milling process. Thereby bitter taste notes may be at least partly diminished in the final galactomannan product.

In further embodiments of the invention the salts in the wetting medium are selected from the group consisting of sodium salts, potassium salts, calcium salts, barium salts, copper salts, magnesium salts, iron salts and any combination thereof.
It is advantageous to use non-toxic salts in the wetting medium.
Preferred salts are e.g. NaCl, KC1 and/or CaCl₂.

According to a further embodiment of the invention the infused fenugreek seeds are frozen and thawed prior to extraction.

**DETAILED DESCRIPTION**

In the following, the extraction process according to the present invention is described in more detail. The galactomannans obtained from fenugreek seeds according to the present invention are of high quality and are useful in many applications.
Because of the high purity (white colour, neutral taste) and the basic features of hydrocolloids, the galactomannans obtained according to the disclosed extraction method can be used in basic compositions for food and cosmetics.

Galactomannans from Fenugreek seed do not split milk making them especially useful as additives to diary products.
Furthermore there is increasing evidence that galactomannans from fenugreek seeds have useful medicinal applications, e.g. in the reduction of cholesterol levels in human blood. In the case of pharmaceutical applications, the selectivity and reproducibility of the extraction process and the purity of the galactomanns obtained is particularly important.
In the following, specific embodiments of the present invention are disclosed by examples 1 - 4. Examples 2 and 3 are comparative example, the extraction being run at a pH outside the range disclosed in claim 1.

EXAMPLES

Example 1

50 g of air-cleaned whole fenugreek seeds are immersed in 5 % NaCl-solution and the pH is adjusted to about 3 with glacial acetic acid while stirring the solution. The temperature of the solution is kept at about 40 °C throughout the extraction. The solution is periodically stirred during the first 4 hours of extraction and stirring is maintained at about 300 rpm for the last 4 hours of extraction.

First, the volume of the seeds increases, because the galactomannans in the seeds take up water. Then, the seed coat begins to break and galactomannans are released into the solution. The seed coat and the cotyledons separate. If stirring is too vigorous, the cotyledons will break up.

After a total of about 8 hours of extraction, the extraction mixture is sieved, thereby separating the solid parts, retained on the sieve, and the liquid phase containing the desired galactomannans, passing through the sieve.

To the galactomannan solution, IPA-spirit (a blend of 90% ethanol and 10% isopropanol) is added in an amount of 3 times the volume of the filtrate while stirring. The deposited gum comprising galactomannans is collected with a spoon into a beaker. Most of the liquid that runs out from the gum is drained away. The gum is re-dissolved in clean water and then deposited with a new portion of IPA-spirit.

The steps of dissolving and depositing the gum are carried out at least three times in total to obtain a product whiter in appearance and substantially free of salts and acid. The seed mass is washed with 5% salt solution, until all gum is removed from the seeds. The gum product is dried in a hot air oven at 30 - 50 °C for 3 - 8 hours. A yield of 10 g is obtained. The dried galactomannan gum is white, odourless and has a neutral taste. The obtained galactomannans have good solubility in water.
Comparative example 2
Whole fenugreek seeds are treated according to example 1, except that the pH is kept at 11, adjusted with NaOH.

A strong yellow colour is released from the seed into the solution within a few minutes. The released gum becomes yellow as a consequence, and the colour can not be removed in the washing steps.

The dried gum product has a yellow colour and slightly bitter taste.

The colour intensity diminishes, if during the extraction, the pH is adjusted to about 3 with an acid, but the colour does not disappear completely.

Even when the pH is kept around 7 and the conditions of example 1 are used, the obtained gum product has yellow-brown colour and non-neutral taste.

Comparative example 3
Whole fenugreek seeds (50 g) are immersed in a solution at pH 3, but with no salt added to the solution.

The solution is kept at 40 °C and intermittently slowly stirred.

The seeds swell, but no gum is released after several hours.

Example 4
500 g of Fenugreek seeds were infused with a solution of 4% CaCl₂ overnight and wet milled using fresh solution of 4% CaCl₂ as the wetting medium. The fenugreek seed mass is slimy and blocks the mill. The mill has to be cleaned several times during the process to remove the blocking masses. The process is slowed down because of the blockages.

After milling the seeds became a powder - this means that all cell content was mixed together and the result was a porridge mixture. The powder was immersed in an extraction medium according to Example 1 and the extraction was performed according to the process of Example 1. To isolate the gum, the solution was passed through a conventional filter but during filtration severe filter blocking was experienced. The gum was deposited according to the procedures in Example 1. The
product yield was small and the colour grey, indicating impurities like protein and starches. Better results could probably be achieved using an ultra-centrifuge for separation of solids from the extracted gum.

5 Evaluation of the Examples

By comparing the results of Examples 1 and 3 it becomes evident that it is the combination of acidic pH and salts in the extraction solution that accomplishes the selective extraction of galactomannans from whole fenugreek seeds. Galactomannan is not released from the whole seeds at acidic pH alone. It has surprisingly been found by the inventor that salt is needed to accomplish the extraction.

By comparison between the galactomannan products from Example 1 and comparative Example 2, it becomes clear that to obtain a superior galactomannan product with respect to purity and overall sensory quality, acidic pH is preferable over basic pH in the extraction medium. Keeping the pH low, beneath about 5, in the extraction solution ensures minimal discolouration and more neutral taste of the galactomannan product.

The solids collected after extraction according to the present invention further contain valuable products that may be obtained through further process steps. In particular, when whole seeds are used for the extraction, more or less intact fenugreek seeds may be obtained which are particularly suitable for use in further extraction steps, from which valuable products, e.g. oils and proteins, may be obtained.

Example 4 shows that the extraction process according to the present invention can also be used on milled seeds, in this case, fenugreek seeds subjected to wet milling after infusion in a salt solution overnight. The extraction process becomes more troublesome in that the separation of the solids and the solution containing the extracted gum is not as straightforward as when using whole seeds like in Example 1. Also, because the seeds are destroyed when milled, whereby the whole seed content is subjected to the extraction medium, the gum product obtained is not as pure as when using whole seeds for the extraction.
Nevertheless, the synergy between low pH and the addition of salts to the solution for extraction may still apply when using milled or otherwise crushed or processed seeds, but more purification steps and more sophisticated separation equipment may be needed to obtain a sufficiently well defined galactomannan product.

According to the present invention and as evidenced by the above Examples, a very simple and versatile method for the extraction of galactomannans from fenugreek seeds is disclosed. The mild process conditions, cheap process chemicals, a simple overall process lay out and products of superior quality solve several problems encountered when using prior art extraction processes.
CLAIMS

1. Method of extracting galactomannans from fenugreek seeds comprising the steps of:
preparing a solution of one or more salts in water,
said salts being present in an amount of 0.5 - 10% by weight of the solution,
adjusting the pH in the solution to be in the range of 1 to 5 with an acid,
keeping the temperature of the solution in the range of 10 - 60 °C,
immersing the fenugreek seeds in the solution for between 2 to 72 hours,
recovering the galactomannans from the solution and
recovering the fenugreek seeds for further processing.

2. Method according to claim 1, wherein
the salts are selected from the group consisting of sodium salts, potassium salts,
calcium salts barium salts, copper salts, magnesium salts, iron salts and any combination thereof.

3. Method according to claim 1 or 2, wherein
the acid is selected from the group consisting of acetic acid, hydrochloric acid, nitric acid and sulphuric acid.

4. Method according to any of the claims 1-3, wherein the step of recovering the galactomannans from the solution comprises the steps of
depositing the galactomannans with a polar alcohol,
re-dissolving the galactomannans in water at least once,
repeating said depositing of the galactomannans and
drying the galactomannans.

5. Method according to any of the claims 1-4, wherein the pH is in the range of 2 - 4,
preferably in the range of 2.5 - 3.5.
6. Method according to any of the claims 1-5, wherein the temperature of the solution is in the range of 20-50 °C, preferably in the range of 30-40 °C.

7. Method according to any of the claims 1-6, said method comprising the step of infusing the fenugreek seeds with a solution of one or more salts prior to the step of immersing the fenugreek seeds in the acidic salt-containing solution.

8. Method according to claim 7, wherein the salts in the solution are selected from the group consisting of sodium salts, potassium salts, calcium salts barium salts, copper salts, magnesium salts, iron salts and any combination thereof.

9. Method according to any of the claims 1-7, wherein the fenugreek seeds are used as whole seeds in the extraction.

10. Method according to any of the claims 1-7, wherein the fenugreek seeds are dry milled prior to extraction.

11. Method according to any of the claims 1-7, wherein the fenugreek seeds are wet milled prior to extraction.

12. Method according to claim 10, wherein the wetting medium is a solution of one or more salts.

13. Method according to claim 12, wherein the salts in the wetting medium are selected from the group consisting of sodium salts, potassium salts, calcium salts barium salts, copper salts, magnesium salts, iron salts and any combination thereof.

14. Method according to claim 7, wherein the infused fenugreek seeds are frozen and thawed prior to extraction.
15. Method according to any of the claims 1, 7 and 12, wherein the one or more salts are selected from the group of NaCl, KCl and CaCl₂.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23L1/052 A23L1/212 A61K36/185 A61K36/48

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)
A23L A61K

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

Electronic database consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Relevant to claim No.</th>
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<td>WO 2009/057125 AI (AVESTHAGEN LTD [IN] ; PATELL VILLO0 MORAWALA [IN] ; HENJARAPPA JAGADEESH) 7 May 2009 (2009-05-07) cited in the application on * examples 1 and 2; claims 15-38 *</td>
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<tr>
<td>A</td>
<td>EP 0 775 451 AI (VITAMED REMEDIES PRIVATE LIMIT [IN]) 28 May 1997 (1997-05-28) cited in the application on * claims 1-10 *</td>
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Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search
8 October 2010

Date of mailing of the international search report
18/10/2010

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<td>30-09-2010</td>
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<td>US 5658571 A</td>
<td>19-08-1997</td>
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