7.1 Other Protein Sweetener - MilisBio’s new protein sweetener **

(Rank 5)

Back to Ranking

Solution

MilisBio's new protein sweetener (1/4)

Application in food & beverages.

The solution to the problem is invented by MilisBio

Reporting period: 2018-05-01 to 2018-09-30

- **MilisBio is developing a sugar substitute from protein that will satiate our cravings without harming our health.**
- There is currently no widely available sweetener without at least suspected associated health concerns. In time the company intend to apply the same method of protein design to other flavours such as saltiness without salt, bitter taste blockers for medicines and so on. MilisBio is a private company founded in 2016 and located in Ireland, Europe. Michael Sheehan is the CEO and Co-founder of MilisBio. **The company is presently developing a proof of concept sweet protein, using technology which will in the future be adapted to different flavors. MilisBio is designing the next generation of flavorings. The ingredients designed by the company's technology is low calorie and naturally digested proteins, designed bespoke to the needs of each customer.** For e.g. A beverage producer can have a high potency, water soluble ingredient. A baked goods producer can have a low potency, heat tolerant ingredient. And so on. MilisBio began in May of 2016, in the RebelBio accelerator program run by SOS Ventures. This developed the R&D strategy, that connected the company to the world of biotechnology and set their work in motion. The company joined the Startup Bootcamp Foodtech program in November of 2016, forging connections with the food industry and mentors specifically experienced in that space, while developing their business model. From September 2017 to September 2018, MilisBio participated in the University College Cork IGNITE Incubator program. This gave the company the time, workshops and mentorship to build a comprehensive plan for their future, and a reservoir of invaluable connections.
- Sweet proteins that occur naturally in some tropical plants can have a sweetness a thousand times that of sucrose. This indicates that proteins potentially represent novel low-calorie, nutritious sweeteners – superior to either sugar or artificial sweeteners. **MilisBio developed "Milis", a novel protein which will be 200 - 500 times sweeter than sugar per gram. Milis presents low caloric content, no aftertaste, and no unhealthy chemical components. Milis allows the consumer to think only about how great their food tastes, rather than worrying about what's in it. Using a protein as a flavoring guarantees that the ingredient will be low-calorie, easily digestible, and suitable for diabetics.**
- **The startup is using screening technology, the method of detecting and separating out different types of proteins, to develop potential flavorings. The firm starts with a protein library (billion proteins) that has those (sweet) qualities and then it is screened.**
- **The startup hopes the screening will identify marketable proteins to act as sweeteners from the large initial pool of candidate.**
- **Using a protein based ingredient with this method guarantees that the ingredient will be low-calorie, high protein, easily digestible and without aftertaste.**
- Throughout the project, Milis Bio has greatly expanded their knowledge regarding the necessary steps to being their proteins to market.

Source1 Source2

Back to Contents

MilisBio's new protein sweetener (2/4)

Applications

- The innovation in MilisBio has the ability to develop lots of proteins at the same time.
- The massive market of soft drinks is one segment of the food and drinks that have the immense business potential. Drinks producers in Ireland were hit with a new sugar tax, forcing the industry to react. Many firms have already cut the sugar content in their products to avoid being hit with the levy.
- Expansion of MilisBio's portfolio to create proteins that also substitute other flavors like saltiness or bitterness.

Conventional Solutions	Advantages
The existing protein sweeteners lack one or the other quality.	The novel sweetener is highly potent in terms of sweetness, water soluble, heat tolerant, and has no aftertaste.
No current technology existed to screen naturally occurring proteins that can replace sweeteners and flavorings.	Screening process allows proteins designed for specific needs meeting the exact needs of clients.

Comments

- With cutting edge technology, MilisBio plans to additionally create flavours such as saltiness without salt, bitter taste blockers for medicines and much more.
- MilisBio is creating a process that will efficiently produce low calorie, non artificial, high potency ingredients entirely made of proteins. The proteins will have flexibility of other characteristics too, with variable heat resistance, pH tolerance, water or oil solubility, and other characteristics, allowing the company to generate bespoke products to suit different industrial demands of food processing.

Source1 Source2 Source3

Back to Contents

7.2 Other Protein Sweetener - Rapeseed protein Napin **

(Rank 10)

Back to Ranking

Sweet rapeseed protein - Napin (1/4)

The solution to the problem is invented by DSM

Patent Published in 2021

Solution

- **The reference discloses about a process for obtaining a native rapeseed protein isolate comprising:**
- **Mixing cold-pressed rapeseed oil meal with an aqueous liquid at a temperature of from 45 to 65° C.**
- Separation of the aqueous liquid from the mixture obtained.
- Decreaming of the aqueous liquid obtained.
- **Adjusting the pH of the decreamed aqueous liquid obtained, to neutral by adding acid or base, and mixing with a precipitant to obtain a precipitate wherein said precipitant comprises a salt of magnesium, zinc, iron, or calcium.**
- Removing the precipitate obtained to obtain an aqueous liquid.
- Concentrating and washing the aqueous liquid obtained.
- **Subjecting the concentrated and washed aqueous liquid obtained to filtration over a membrane with a cut off ⟩ 50 kDa.**
- **Subjecting the permeate obtained to filtration over a membrane with a cut off between 5 and 50 kDa.**
- **Isolating native rapeseed protein isolate from the concentrated and washed aqueous retentate obtained by means of drying.**
- **wherein ascorbic acid or a derivative thereof and a sulfite is added before, during or after any of the above mentioned steps.**

Application in food & beverages.

- **Native rapeseed protein isolate comprising more than 60 wt. % napins and from 30 to 3,000 mg/kg of phenolics.**
- **Native rapeseed protein isolate has a 2 wt. % solution in water, a sweetness equivalent to an aqueous sucrose solution of at least 10 g/L.**
- **The native rapeseed protein isolate of the invention has a solubility of at least 88%, preferably at least 90%, more preferably at least 94%, and most preferably at least 96%, at a pH in the range of from 3 to 10 at a temperature of 23±2° C.**
- **The isolate can be used in food products such as a beverage, confectionary product, health bar, chocolate, or a milk powder.**

Source

Back to Contents

Sweet rapeseed protein - Napin (2/4)

Tests

Protein Content: Protein content was determined by the Kjeldahl method, AOAC Official Method 991.20 Nitrogen (Total) in Milk, using a conversion factor of 6.25, to determine the amount of protein (wt %).

Color Measurement Using UV-Spectrophotometer: Color values were determined using an UV-spectrophotometer (TECAN Infinite M1000 Pro plate reader) with 96-wells plates. The sample volume per well was 275 µl. Samples were clarified by filtration (0.45 µm) before absorbance measurements. **Measured absorbance at 400-700 nm** (10 nm interval, corrected for blank (MilliQ water)) was converted to L values using the formulas as described in DIN 5033 Part 3 and DIN 6174. For the calculation of L, illuminant D65 was used and the "CIE 1964 supplementary standard colorimetric observer" standard spectral functions with an observer angle of 10°. For comparison of 100-L between different samples, extrapolated 100-L values were used since L (or 100-L) does not have a linear relationship with sample concentration.

Phenolics Content: Pretreatment standard: approximately 10 mg sinapic acid standard (Aldrich), was weighed accurately to 0.01 mg and dissolved in 50.0 mL of an aqueous solution of methanol (50%) and acetic acid (0.5%). Pretreatment rapeseed protein isolate samples: About 1 g of sample was weighed in a 50 mL Greiner tube and diluted with 9 mL of an aqueous solution of methanol (50%) and acetic acid (0.5%). Samples were shaken for about 60 minutes at 2,000 rpm and maintained overnight at 4° C. after which samples were centrifuged (4,500 rpm, 10 min, 4° C.). 1 mL of the supernatant was transferred to a 2 mL Eppendorf tube and centrifuged again (14,000 rpm, 10 min, 4° C.). 0.5 mL of the supernatant was analyzed in the above HPLC procedure.

Conductivity: The conductivity of native rapeseed protein isolate in a 2 wt. % aqueous solution was measured using a conductivity meter.

Solubility Test: The below solubility test is adapted from Morr et al., the difference being the use of water instead of 0.1 M sodium chloride.

MW Determination by Blue Native PAGE: In the case of Native PAGE, the protein charge has an impact on the electrophoretic mobility. In the case of Blue native PAGE (and to the contrary of clear native PAGE), the Coomassie Brilliant Blue dye provides the necessary charges to the protein complexes for the electrophoretic separation.

Phytate Level: Phytates were measured at Eurofins using method QD495

Although the meal has a relative high protein content, **in the prior arts, the quality of the proteins is reduced significantly resulting from the harsh conditions (i.e. , elevated temperature, solvents) employed during the oil extraction**. In present invention , the final step of processing, **the washed concentrate may be dried in a suitable dryer**, such as a spray drier (single or multistage) **with an inlet temperature in the range of from 150 to 200 °C. and an outlet temperature in the range of from 50 to 100 ° C. resulting in the rapeseed protein isolate**.

Source

Back to Contents

Sweet rapeseed protein - Napin (3/4)

Results

Conductivity: The conductivity of the resultant native rapeseed **protein isolates in a 2% solution was less than 4,000 µS/cm over a pH range of 2.5 to 11.5.**

MW Determination by Blue Native PAGE: Blue Native PAGE: **Main bands were observed roughly around 300 kDa, between the 242 and 480 kDa MW markers. Some staining was visible as a smear as lower MW (150 kDa and below). No clear bands were observed at 150 kDa.** Based on these results, the rapeseed product contains the 12S form of cruciferin. The resultant native rapeseed protein isolate comprised in the range of from 40 to 65% cruciferins and 35 to 60% napins.

Phenolic and Phylate Content: The resultant native rapeseed protein isolate contained less than 0.26 wt. % phytate and had an amount of phenolics of 3,500-7,400 mg/kg.

Solubility Test: The resultant native rapeseed protein isolates had a solubility of at least 88% when measured over a pH range from 3 to 10 at a temperature of 23±2° C.

pH	3	4	5	6	7	8	9	10
Sample 1 Solubility (%)	98	96	89	95	95	97	97	98
Sample 2 Solubility (%)	102.5	97.5	94.3	93.9	97.0	93.0	94.0	99.8

- **The rapeseed protein isolate is dried at 150 to 200 °C** indicates that the protein isolate is stable at this temperature**.**
- The method is applicable for producing rapeseed protein isolate having sweetening effect with **pH stability, greater solubility**.

Eight persons evaluated the protein solutions , comparing the three solutions and ranking them according to sweetness as outlined in the below Table.

Code	2% aqueous solution	Sensory description of sweetness
A	Native rapeseed protein isolate sample 1 (of Comparative Example 1)	Slightly sweet; less sweet than C
B	Fraction I (Example 3)	Not sweet
C	Fraction II (Example 3)	Sweet; significantly sweeter than A

Number of panelists scoring sweetness of a 2% aqueous solution of native rapeseed protein isolate of the present invention versus reference sucrose solutions (n = 5)

	Fraction II is less sweet	Fraction II has similar sweetness	Fraction II is sweeter
Sucrose 1.5%	0	0	5
Sucrose 2%	0	0	5
Sucrose 3%	1	2	2
Sucrose 4%	4	0	1
Sucrose 5%	5	0	0

Advantages

The method increased the thermal stability, pH stability, solubility of the sweet proteins.

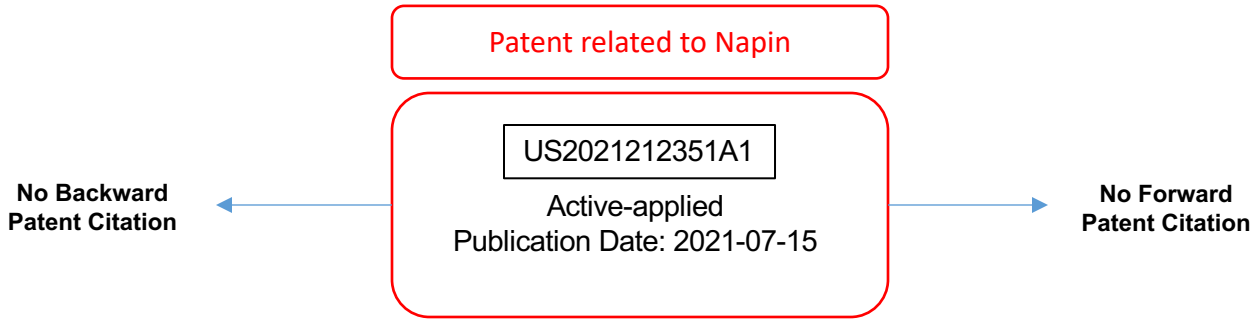
Source

Back to Contents

Sweet rapeseed protein – Napin (4/4)

Citation Tree

❑ **Citation tree helps to understand the strength of the technology based on count of companies or universities taking help form the inventive technology.**



Backward Citations are earlier published documents that are publicly available before the filing date of the new patent application, also called "prior art". These can be cited by an applicant, third party or a patent office examiner because of its content relates to the new patent application.

Forward citations are more recently published documents which has gone on to cite the new patent application. These can be cited by an applicant, third party or a patent office examiner because of its content relates to the new patent

Note that citation tree has been provided for top ten ranked solutions only.

Back to Contents