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Formulation Development of Antimicrobial Gel Using Chia Seed Mucilage

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Abstract

The present study was aimed to investigate antimicrobial potential of gel formulated with ***Betel leaf*** extract and ***Chia seed Mucilage*** extract against two gram positive bacteria, two gram negative bacteria and antimicrobial strain. Different gels were formulated by varying the concentration of the extracts and evaluation was done by cup plate method for zone of inhibition and broth dilution method for MIC (Minimum Inhibitory Concentration) determination. The formulated gel was compared for antimicrobial activity with standard marketed silver sulfadiazine preparation. MIC range for *Betel Leaf* and *chia seed Mucilage* was found to be 0.05- 3.2 mg ml^{-1} and 5-25 mg ml^{-1} respectively. Formulation containing these extracts, showed significant zone of inhibition for 0.25%, 0.5%, 1%, 2%, 3% of which 1% showed maximum zone of inhibition (ranging from 30.5 to 37 mm) as compared to marketed preparation. The selected 1% gel formulation also showed good Antimicrobial activity when compared to marketed preparation. Thus the present investigation revealed that the developed gel formulation has potential antimicrobial activity.

Keywords: Betel Leaf, Chia Seed Mucilage, Zone of Inhibition.MIC, Antimicrobial Activity.

1. INTRODUCTION

Gels are semisolid systems in which a liquid phase is constrained within a three dimensional polymeric matrix of natural or synthetic gums in which a high degree of physical or chemical cross linking has been established (Kaur P., 2013). The U.S.P. defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. (Kaur L., 2013).

Gels consist of two-phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved in the continuous phase (Chandel A., 2013).

Most topical gels are prepared with organic polymers, such as Chia seed Mucilage that impart an aesthetically pleasing, clear, sparkling appearance to the products and are easily washed off from the skin with water. The type of base used in formulating a topical dermatological product greatly influences its effectiveness (Bharadwaj S., 2012).

Advantages of Gel:

- Stable Complex
- Film Forming Capacity
- Prolonged Germicidal Action
- Adheres to treated surface
- Water soluble hence eases to remove or wash

Uses of gels:

The use of gels and gelling agents are quite widespread, even in limiting our consideration to the pharmaceutical and cosmetic field only. Gels find use as delivery system for oral administration as gels proper or as capsules shells made from gelatin.

Gelling agents are useful as binder in tablet granulation, protective colloidal in suspensions, thickeners in oral liquids and suppository bases. Cosmetically, gels have been employed in a wide variety of products, including shampoos, fragrance products, skin and hair care preparations.

Properties of gel:

- For pharmaceutical or cosmetic application the gelling agent should be safe, inert and it should not react with other formulation components.
- The gelling agent must be included in the preparation to create reasonable solid-like nature during storage that can be easily broken when subjected to shear forces generated by squeezing the tube, or during topical application.
- It must contain relevant anti-microbial property to prevent from microbial attack.
- The topical gel must not be sticky.

Types of gel:

a. Hydrogels: Hydrogel is a system of polymer chains that are hydrophilic, once in a while found as a colloidal gel in which water is the scattering medium. Hydrogels are exceedingly absorbent (they can contain over 99.9% water normal or engineered polymers). Hydrogels likewise have a level of adaptability fundamentally the same as regular tissue, because of their critical water content. Regular uses for hydrogels incorporate;
   - Currently utilized as platforms in tissue building. At the point when utilized as platforms, hydrogels may
   - contain human cells to cure tissue.
   - Hydrogel-covered wells have been utilized for cell culture
   - Environmentally delicate hydrogels which are called 'Smart Gels' or 'Intelligent Gels'. These hydrogels can detect changes of pH, temperature

or the convergence of metabolite and discharge their heap as after effect of such a change.
- As continued discharge tranquilize conveyance frameworks.
- Provide retention, DE sloughing and debriding of necrotic and fibrotic
- Used in dispensable diapers where they retain urine, or in sterile napkins.
- Contact lenses (silicone hydrogels, polyacrylamides, polymacon).
- Rectal medicate conveyance and determination.

b. Organogel: An organogel is a non-crystalline ,non-smooth thermo reversible (thermoplastic) strong material made out of a liquid organic stage captured in a three dimensionally cross-connected system. The fluid can be a natural solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structurants are significant qualities for the elastic properties and solidness of the organogel. Regularly, these frameworks depend on self-assembly of the structurant atoms. Organogels have potential for use in various applications, for example, in pharmaceuticals, beauty care products, art preservation, and sustenance. A case of arrangement of an undesired thermo reversible system is the event of wax crystallization in oil.

c. Xerogel : A xerogel is a solid structure from a gel by drying with unhindered shrinkage. Xerogels ordinarily hold high porosity (15–half) and tremendous surface region (150–900 m2/g), alongside small pore size (1–10 nm). At the point when solvent removal happens under harsh (super critical) conditions, the system does not shrink and a profoundly permeable, low-thickness material known as an aerogel is delivered. Warmth treatment of a xerogel at raised temperature produces gooey sintering (shrinkage of the xerogel because of a limited quantity of thick stream) and viably changes the permeable gel into a thick glass. Polymers are used to give the auxiliary system, which is basic for the arrangement of gels.

Introduction Carbopol 934 P

Carbopol is made of carbomers. Carbomer polymers are cross-linked together and make a microgel structure that makes them optimal to be used as a drug vehicle for dermatological purposes. They can be used in cases when drug delivery in a controlled manner is desired. The microgel structure makes it possible for these systems to tolerate the physical movement of the body and shape themselves after the application area movement (Islam, *et al.* 2004). Carbopol polymers are acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. These polymers are anionic polymers that need naturalization to become gellified. Organic amines like triethanolamine can be used to naturalize these polymers in liquids (Islam et al. 2004). Carbopol polymers have been used in the personal care industry for forty years. They have been used in producing gels, creams, lotions and suntan products. Carbopol gels have been applied as drug vehicles in several routes of administration.

CHIA SEED

Functional Category: Gelling agent, stabilizing agents, Thickening agent

DEFINITON

Mucilage is a long chain Polysaccharide substance extracted as a viscus or of gelatinous solution from plant part (roots, seed, leaves etc.) That are hydrophilic, being able to attract bind with volume of water that far exceeds the mass of the mucilage. They are biocompatible and non-toxic in nature.

SOURCE:

- Root-Satavari mucilage (Asparagus racemosus, Apocynaceae).
- Seed-Chia seed (Salvia hispanica, Lamiaceae).
- SeehHusk-Ispagol (Plantagonpsyllium,Plantaginaceae).
- Leaves-Sisi leaves (Cocculus hirstus,Menispermaceae).

PHARMACEUTICAL USES:

Tablet binders, disintegrant, emulsifying and suspending agents in biphasic liquid dosage form.

Gelling agents, stabilizing agents, thickening agents, film forming agents in transdermal and periodontal films, buccal tablet, sustaining agents in matrix tablets and coating agents in microcapsules including those used for protines delivery.

Chia is an edible seed that comes from the desert plant Salvia hispanica, grown in Mexico where they were highly valued for their medicinal properties and nutritional value. They are unprocessed, whole-grain food that can be absorbed by the body as seeds (unlike flaxseeds).

One ounce (about 2 tablespoons) contains 139 calories, 4 grams of protein, 9 grams fat, 12 grams carbohydrates and 11 grams of fiber, plus vitamins and minerals and antioxidants.

2. MATERIALS AND METHODS

The investigation included the definition and improvement of home grown antimicrobial gel containing Betel leaf and Curcumin and Betel leaf. Concentrate Formulation of gel were set up by taking various groupings of Carbopol 934 and Xanthan gum separately. Best upgraded cluster was chosen for definition of gel .Different details were plan dusing changing measure of gelling specialist. The strategy just contrasted in procedure of making gel in various definitions. The arrangement of gel was same in all plan The gel stage in the definitions was set up by scattering Carbopol 934 (1g) and Xanthan gum in cleaned water (50 ml) with steady blending at a moderate speed utilizing mechanical shaker, at that point the pH was acclimated to 5.0-7.4 utilizing triethanolamine (TEA).Methyl paraben (0.03g) were broken down in propylene glycol (5g) and were blended with the fluid stage. At that point fluid stage with constant mixing until it progresses toward becoming cooled to room temperature. The acquired gel was blended with in 1:1 proportion with delicate mixing to get the gel.

Sr.no	Ingredient	HG1	HG2	HG3	HG4	HG5	HG6
1.	Betel Leaf (extract mg)	100	100	100	100	100	100
2.	Curcumin (extract mg)	100	100	100	100	100	100
3.	Chia Seed Mucilage (extract)	0.5	1.0	1.5	2.0	2.5	30
4.	Carbopol 934 (mg)	0.5	1.0	1.5	2.0	2.5	3.0
5.	Xanthan gum(mg)	0.5	1.0	1.5	2.0	2.5	3.0
6.	Polyethylene glycol(ml)	10	10	10	10	10	10
7.	Methyl paraben(g)	0.08	0.08	0.08	0.08	0.08	0.08

a) Determination of melting point

The melting point of extract was measured using capillary method by using melting point apparatus.

b) Spectral analysis of herbal extract λmax

Accurately weighed (10 mg) extract was transferred to a previously dried 100ml flask and dissolve dousing little quantity on DMSO.this solution was sonicated and shakenfor15min. This solution was filtered using particular filter paper. The solution was taken in 20 ml volumetric flask and diluted up to the mark. To get a stock solution of 1000 ppm.it was filtered and further diluted to get a solution of concentration 10ppm & was scanned intherange of 400-800 nm using methanol as a blank. The point in absorption spectra at which extract showed maximum absorption was recorded as a λmax (1800, Shimadzu, Japan) at 246 nm against blank.

Identification of extract by FT-IRspectroscopy:

The IR spectrum of extract was measured by using FT-IR spectrophotometer. Fourier transform infrared (FT-IR) was used to identify the characteristic functional group in the sample. A small quantity of sample was scanned over a range of 4000-400 cm -1 in FTIR instrument (Alpha Brucker, Germany).

c) Drug- excipient compatibility study:

The interaction of drug and polymer round experimental condition is an important prerequisite before formulation .It is necessary to confirm that the drug extract does not with the polymer or excipient and affect the shelf life of the product this can be confirmed by carrying out by FT-IR studies.

Evaluation of gel formulation :

a) Physical appearance

The formulation of herbal hair gel was evaluated for organoleptic characteristics, visual appearance, odour, color, texture, consistency.

b) Measurement ofpH

The pH of the formulated gel was determined using digital pH meter (Systronics Instruments). The electrode was immersed in the gel and readings were recorded from pH meter.

c) Washability

A specific quantity of gel was scrubed on the skin of the back of the hand, and then gel was washed with water and observed whether it is washable or noted.

d) Determination Viscosity

Consistency estimations were done on Brookfield viscometer (LV DVE, Brookfield Engineering Corporation, USA) by choosing axle number 63 and speed 50 rpm. Gel (50 g) was kept in 50 ml recepticle which was set till shaft section was plunged and dialed perusing estimated following three minutes. From the perusing got, consistency was determined by utilizing factor. The strategy was rehashed multiple times and perceptions were recorded as mean ± SD.

e) Drug content (gel)

1.0 g of gel details was taken in 100 ml volumetric cup containing 20 ml of DMSO and blended for 30 minutes permitted to represent 24 hours if there should arise an occurrence of natural antimicrobial gel definitions. The resultant arrangement was sifted through layer channel. The absorbance of the arrangement was estimated spectrophotometric partner at 435 nm (Betel leaf) and 470 nm (curcuma longa leaf) utilizing antimicrobial gel arrangement as reference (Kedor-Hackmann et al., 2006).

f) Texture profile analysis

Surface profile examination (TPA) was performed utilizing a Brookfield CT3 Texture Analyzer in pressure mode by utilizing spread capacity test frill (TA-SF).gel Formulation HG1-HG6 was filled into the female test, taking consideration to keep away from air pocket into the examples. A funnel shaped investigative male probe(35mm distance across of 45°)was constrained down into each example at a characterized rate (1 mm/s) and to a characterized profundity (10 mm). In any event two repeat investigations of test were performed at temperature 350°c. From the subsequent power time plots, the hardness (the power required to achieve given distortion), cohesiveness (the antimicrobial gel in down development of test) and adhesiveness (the work important to beat the appealing powers between the outside of the example and the outside of the test) were inferred. Spread capacity was determined from the vitality required to distort the example or from the hardness of the example.

g) Homogeneity and grittiness

h) A little amount of gel was squeezed between the thumb and the forefinger (regardless of whether homogeneous or not). Additionally, the homogeneity can be identified when a little amount of the gel was scoured on the skin. The dirt of arranged gel is additionally seen in the equivalent manner(Hotkaretal2013, Loydetal2011,Karasuluetal,2006; Keithetal,1998).

i) In vitro diffusion study

In vitro medication discharge study was done utilizing Franz dissemination cell with a receptor compartment(25ml) and a viable dispersion region of 3.14cm².Cellulose dialysis film 150 LA401-1MT (Himedia, Mumbai, India) was absorbed receptor media

(phosphate cushion, pH 5.0) for 24 h before the investigation. A foreordained measure of gel home grown was set on the giver side. The receptor medium was ceaselessly blended at 350 rpm and thermo expressed at 37±0.5°C with a water coat. At foreordained time interims, 5 ml tests were pulled back from the beneficiary compartment and time interim of 0, 15, 30, 45, 60, 90, 120, 140, 160, and so forth supplanted with an equivalent volume of crisp cradle. The gathered examples were examined by UV spectrophotometer.

j) Stability ponders

The readied home grown gel was pressed in holder (5 g) and exposed to security concentrates as indicated by ICH rules at 25±2°C/60±5% RH for a time of multi month. Tests were pulled back at 15-day time interims and assessed for physical appearance, scent, pH, rheological properties and medication content.

3. Result And Discussions

3.1 Formulation and development:

3.1.1 Preliminary trial batches

Six trial batches were formulated and evaluated for pH, viscosity, appearance and spreading coefficient. Theses trial batch have very high spreading coefficient which can be attributed to concentrations of Carbopol 934 and Xanthan gum, propylene glycol.

Table 3.1.1 .Preliminary optimization of herbal gel

Sr.no	Ingredients	HG1	HG2
1.	Carbolpol 934 (g)	1	1
2.	Xanthan gum (g)	1	1
3.	Glycerine (ml)	5	5
4.	Methyl paraben (g)	0.08	0.08
5.	Polyethylene glycol (ml)	10	10
6.	Dmso(ml)	10	10
7.	Triethanolamine (ml)	1.2	1.2
8.	Distilled water (ml)	q.s	q.s

Evaluation of antimicrobial gel:

a) Physical appearance:

Physical evaluation was done to check the appearance, color, odor, solubility, etc.

Table 3.1.2. Physical appearance of gel formulation (HG1-HG6).

Sr.no	Parameters	Gel formulation
1.	Color	Dark green
2.	Odor	Characteritics
3.	Solubility	Soluble in DMSO

b) Measurement of pH:

The pH of formulation of HG1 to HG6 was found to be rang 4.5 to 7. Each sample was analytical in triplicate. The results that formulation provide an acceptable pH in rang of antimicrobial drug 4.5 to 7.0

Table 3.1.3. pH of gel formulation

Sr.no	Formulation code	pH
1.	HG1	7.1
2.	HG2	6.8
3.	HG3	7.3
4.	HG4	6.7
5.	HG5	6.9
6.	HG6	7.0

c) Viscosity :

A viscometer (Brookfield engineering company, USA) was used to viscosities (in cps) of the gels. The viscosity of HG1 to HG6 formulation was found to be in the rang (23521-25112 cps) by using spindle no.63. The results indicated that the good viscosity, was sufficient to apply topically to retain on hair.

Table 3.1.4. Viscosity of gel formulation

Sr.no	Formulation code	Viscosity (cps)
1.	HG1	23521
2.	HG2	20434
3.	HG3	28356
4.	HG4	19826
5.	HG5	21723
6.	HG6	25112

d) Washability:

The washability of formulation HG1 to HG6 show excellent and good washability

Table 3.1.5.Washability of gel formulation

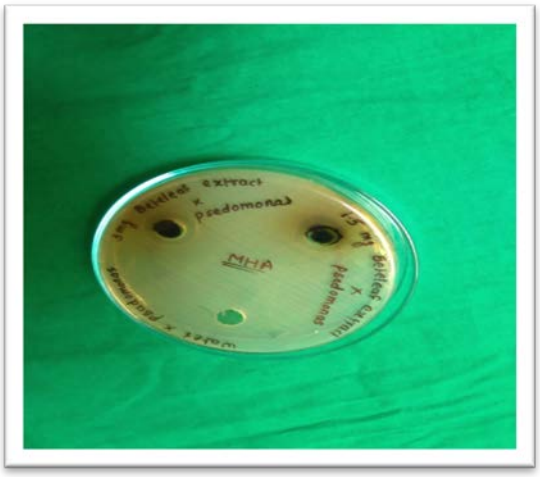
Sr.no	Formulation code	Washability
1.	HG1	++
2.	HG2	+++
3.	HG3	++
4.	HG4	+++
5.	HG5	+++
6.	HG6	++

E) Antimicrobial Activity

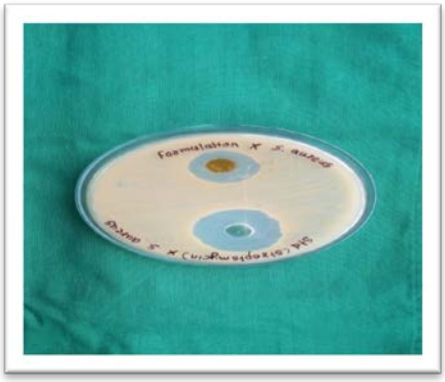
Test Organisms	Product Details – Solvent is DMSO	Zone of inhibition(mm)
Pseudomonas	1.5mg Betel Leaf Extract	16.4 mm
	3.0mg Betel Leaf Extract	18.3 mm
	Water	Nil
Test Organisms	Product Details –Solvent is DMSO	Zone of inhibition(mm)
S.aureus	1.5mg Betel Leaf Extract	16.3 mm
	3.0mg Betel Leaf Extract	19.1 mm
	Water	Nil

Test Organisms	Product Details- Solvent is DMSO	Zone of Inhibition
Pseudomonas	1.5 mg Betel Leaf Extract + 1.5mg Curcumin Extract	18.2
	3 mg Betel Leaf Extract + 3 mg Curcumin Extract	21.8
	DMSO	Nil
Test Organisms	Product Details- Solvent is DMSO	Zone of Inhibition
S.aurues	1.5 mg Betel Leaf Extract + 1.5mg Curcumin Extract	19.5
	3 mg Betel Leaf Extract + 3 mg Curcumin Extract	22.5
	DMSO	Nil

Test Organisms	Product Details – Solvent is DMSO	Zone of inhibition(mm)
Pseudomonas	1.5mg Curcumin Extract	14.9 mm
	3.0mg Curcumin Extract	16.6 mm
	DMSO	Nil
Test Organisms	Product Details – Solvent is DMSO	Zone of inhibition(mm)
S.aureus	1.5mg Curcumin Extract	16.0 mm
	3.0mg Curcumin Extract	18.5mm
	Water	Nil



Final Formulation: Zone of inhibition



f) Texture profile analysis

Texture profile analysis of gel shows the spreadability in term of cohesiveness and adhesiveness. Spreadability denotes the extent of area to which the gel readily spreads on its application. The greater the viscosity, lesser the spreadability (Majithiya *et al.*, 2006) and more is the retention of gel on the skin. Texture profile analysis of the formulated gel (HG 2) showed the hardness of 154 g which

was the maximum force value in the graph. The area under the positive curve is the energy required to deform the sample is 5.3 mJ (hardness work done). The hardness work done and firmness show the spreadability of sample. Higher value of firmness and hardness work indicated less spreadable sample conversely the less value indicate more spreadable sample. The maximum negative force (87g) on the graph indicated sample adhesive force; the more the negative value the more 'sticky' the sample. The area under the negative part of the graph is known as adhesiveness (4.6 mJ) which is the energy required for breaking probe sample contact. These results expressed the retention time of the gel on the site of application.

(g)Stability studies:

Stability studies were performed as per ICH guidelines. The results indicate that there was evident change in the physical appearance color, odor and drug content of formulations after subjecting them to stability studies. Optimized formulation HG2 was chosen for stability studies. At fixed time interval drug content determination of these formulations showed that there were significant changes in the values when compared to the initial formulations. Thus we may conclude that the drug undergo degradation on storage.

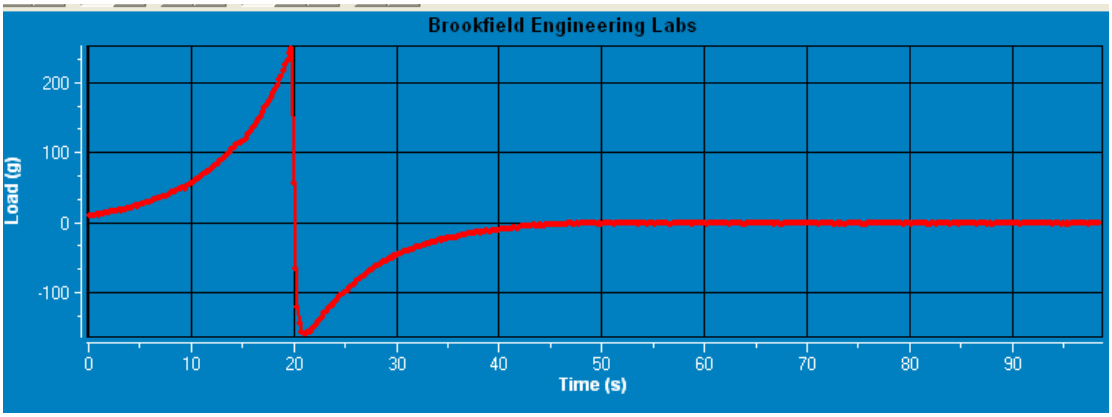


Figure 3.2.1 Texture profile analysis of gel

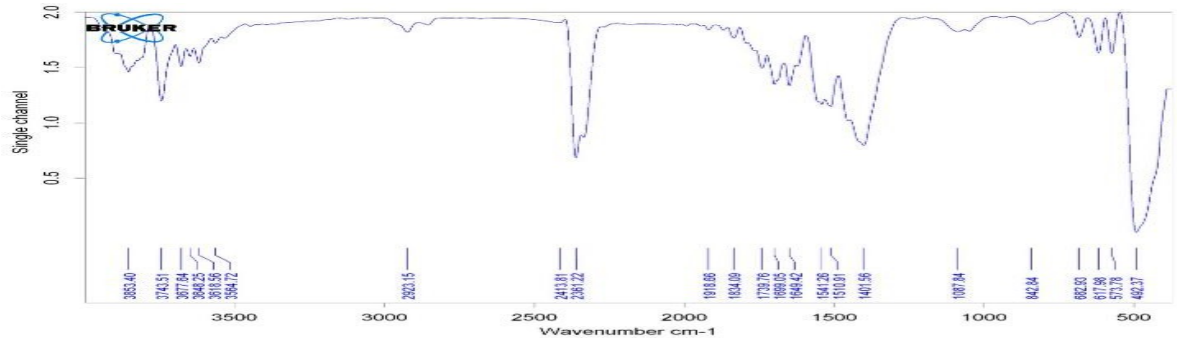


Table 3.2.1 Stability data of optimize batch

Sr.no	Test	Initial observation	Final observation
1.	Appearances	Transparent	Transparent
2.	Color	Dark yellowish	Dark green
3.	Odor	Characteristic	Characteristic
4.	pH	6.82	5.03
5.	Drug content	96.66%	95.73%

4. CONCLUSIONS

Mucilage of chia seeds was located in the outer three layers of the seed coat. When the seed came in contact with water, the mucilage appeared immediately and in a short time it formed a transparent "capsule" surrounding the seed.

The optimum extraction process was performed at a temperature of 80°C with a seed:water ratio of 1:40 (7 % yield). The maximum hydration occurred at low concentrations of salt, pH from 6.5 upwards, reaching a maximum at pH 9, and a temperature from 65 °C with best results at 80°C. The results of this study showed that the mucilage can be easily extracted and hydrated to achieve a water retention of 27 times its weight in water. Chia seeds and mucilage have a great potential as a functional ingredient to be used as thickener in foods.

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