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SKIN CLEANSING BEADS: Comparative Study of β-Glucan from Different Sources for the Production of a Healthy Skin Cleansing Cosmetic Product.

***Shivi Sharma[1], Shivangi Chauhan[2], Pratima Yadav[3] and Amit Mishra [4]**

1. Assistant Professor, Department of Biotechnology, Hindustan College of Science & Technology, Mathura (Affiliated to Dr. .P.J. Abdul Kalam Technical University, Lucknow), Mathura-281122, Uttar Pradesh, India.(+91)
2. B.tech Students of Department of Biotechnology, Hindustan College of Science & Technology, Mathura

Abstract: The present invention relates to a semi-solid composition in the form of cleansing beads comprising β-glucan, omega 3 and vitamin C for use in skin hygiene processes, wherein the brittle material of the beads is ruptured by force by an individual performing a skin hygiene process so that the fragile substance can store the active ingredient until the active ingredient is immediately released from the storage state. The β-glucan and omega 3 are obtained from barley seed and flaxseed. Production of skin cleansers for improving the skin contour by restoring youthful mechanical properties of the skin. More particularly, it relates to semi-solid composition in the form of cleansing beads comprising β-glucan, omega 3 and vitamin C for use in skin hygiene processes.

Index Terms **- β–glucan, Oats, Barley, Omega, Vitamins, alkaline extraction, acidic extraction.**

I. INTRODUCTION

β-glucans is a non- starch polysaccharide that has D-glucose units linked via β-glycosidic bonds. They do not include cellulose, which only has β-1,4 glycosidic bonds. They are mainly formed of β-1,4 and β-1,6 bonds. Because of the presence of β- glucans in the cell walls of many organisms, human beingsunintentionally consume them. There are various sources through which beta glucans can be extracted like cereal grains (mainly oats and barley), mushrooms, seaweeds and yeasts [3].

Beta glucan plays a major significant role in various fields. One of its major role in skin are wound healing, anti-ageing, anti- inflammatory, regenerative effects etc. Beta glucan has pluripotent activity that helps as a complementary activity in managing various skin diseases. They also possess an anti-infective property, wound healing effects, radioprotection and moisturization [1].

For properly understanding all kinds of relations between β-glucan structure, function and the biological effects of dietary have been evaluated after their purification. The differences in the β-glucan preparation procedures under specific experimental conditions result in observation of a limited range of effects. Thus, all type foods of that are having β-glucans is definitely not restricted to the reported effects.

In addition to the above discussion, owning to the different types of difficulties with preparing highly pure and standardized B-glucans, a very careful evaluation, not including the effects of contaminants is required for elucidation of true effects of dietary B-glucans. Various effects on the human body after consumption of dietary β-glucans have been reported, also including the reduction

in the cholesterol level, prevention of diabetes, regulation of the gut microflora, antioxidant activities, anti-tumour effects, and immuno-stimulation. Some important effects are discussed below:

1.1.1 Immuno-stimulation

As we all know, an immune system defends the body against bacterial and viral infections (all other kinds of invaders). A term, known as immunosenescence, is the reduction in the immunological function due to old age. And this as a result, becomes the reason for the increased susceptibility to infections in the elderly. One of the primary causes of immunosenescence is the age-related decline of T-cell function and involution of the thymus.

When a person ingests β-glucan, there is a stimulatory effect on immune homeostasis generated via the β-glucan receptor present in the mucosal immune system and contributes to the prevention of age-related diseases. This effect has been reported for almost all B-glucan containing foods although the effect size may differ.

The immunostimulatory effect of β-glucan is mediated by the innate immunity which has the main purpose of providing defence against pathogens. When a person ingests β-glucans, it affects the mucosal immune system in the gastrointestinal tract. The uptake of microorganisms from the intestinal lumen is tackle by the M cells of Peyer's patches in the small intestine. Subsequently, these cells present the antigen at their basal surfaces to immune cells such as macrophages and dendritic cells.

To identify the pathogens, the receptors specific to pathogens' cell wall are enriched on the surface of immune cells. Several pattern recognition receptors target B-glucans that has existence in the cell wall of microorganisms. In inclusion to acting as a stimulatory signal on cell surface receptors, the microorganisms that pass through the intestinal lumen are phagocytosed by macrophages and have additional immunostimulatory effects. [6]

1.1.2 Immune regulation of the T helper 1 (Th1) / Th2 balance:

The importance of balanced immune homeostasis has been recognized in recent years because of excessive immune responses can lead to autoimmune diseases or allergies. Important example of immune homeostasis is the balance between Th1 cells of cell-mediated immunity and Th2 cells of humoral immunity. The Th1 and Th2-mediated immune reactions supress each other for immunological homeostasis.

Atopic dermatitis represents an example of allergic reactions caused by an imbalance between the cells Th1 and Th2. A change in the immunological equilibrium in the Th2 direction is responsible for the symptoms of atopic dermatitis and other allergies. Meanwhile, excessive activation of the immune system mediated by Th1 cells together with that of Th17 cells causes autoimmune diseases, such as rheumatoid arthritis.

Ingestion of B-glucans primarily activates Th1 and Th17 cells, thereby resulting in the suppression of activity of Th2 cells. This phenomenon shifts the Th1/Th2 response equilibrium in the Thl direction and alleviates the symptoms of allergic diseases. This action is basically triggered by the immunostimulatory effect of B-glucans, suggesting that all B-glucan containing foods possessing immunostimulatory effects also have this activity. [7]

1.1.3 Cholesterol-lowering property

The cholesterol-lowering property of the dietary β-glucan of cereals, like oats and barley, has been known for several decades and has been verified clinically by prescription of dietary β-glucan to patients with high levels of serum cholesterol. The lowering of cholesterol levels does not affect high density lipoprotein (HDL) cholesterol; however, it affects low-density lipoprotein (LDL) cholesterol, which is an important marker of the risk of cardiovascular diseases.

This signifies that consumption of dietary β-glucans present in cereals lowers the risk of cardiovascular diseases. For example, when a person consumes bread formulated with oat β-glucan there is a significant decrease seen in the LDL-cholesterol levels. This is happening because cholesterol-lowering property has only been reported for cereals driven β-glucans. The effects caused by the β-glucan-containing foods on cholesterol levels are still uncertain till date.

The reduction in cholesterol in levels by β-glucan is primarily due to its ability to act as dietary fibre and to entrap the bile acid micelles containing fats. This type of activity disrupts the interaction of micelles with luminal membrane transporters that are present in the intestinal epithelium, which in turn result in the increment of the faecal output of fat, bile acid, and cholesterol. [4]

1.1.4 "Anti-oxidant activity"

Cereals are known to exert anti-oxidant activities by scavenging ROS. In particular, the ROS-scavenging ability of oats and barley mainly derives from their abundant β-glucan, which are known to scavenge ROS aseffectively as other polysaccharides do.

The β-glucan extracted from barley shows a significantly higher anti-oxidant activity as compared to β-glucan from oats and black yeast, indicating that the structure and composition of β-glucan also influence their anti-oxidant activity. Meanwhile, the ROS-scavenging activity of β-glucan -containing foods other than cereals may be low because they include lesser amounts of β-glucan, and its presence is important for this action.

The anti-oxidant effects of ingested cereal β-glucan possibly prevent the damage to the gut that is caused by ROS generated there. In addition to the reports on the physical ability of β-glucan to scavenge ROS, several studies on rats as a model organism have revealed that the anti-oxidant activity of orally administered β-glucan is also directed against oxidative stress induced even in the internal organs such as kidneys and the liver.

These reports imply that β-glucan also exerts the anti-oxidant effect by either of the following mechanisms: β-glucan incorporated into the circulatory system scavenges ROS there, or β-glucan stimulates the innate anti-oxidant system via specific receptors in the mucosal immune system and the subsequent cytokine release. [2]

1.1.5 "Anti-tumour activity"

β-glucan have a great significance and are very important medicinally, such as lentinan, grifolan, and schizophyllan glucan (SPG). And all of these are extracted from different sources, they are as follows:

- Lentinan-Shiitake mushrooms
- Grifolan-Maitake mushrooms
- Schizophyllan-Schizophyllum

All of these above mentioned are used as anti-tumour drugs. The effects of anti-tumour type shown by these drugs are attained by intravenous administration. Though intravenous administration is most effective but administration done orally is recently been developed Superfine dispersed lentinan was seen to show effects on hepatocellular and pancreatic cancers. These studies showed that it is not necessary to administer intravenously, if a person eats mushroom fruit bodies, it is enough to have the potential effects equivalent to anti-tumour drug.

β-glucan show the anti-tumour effects via the β-glucan receptors and is primarily mediated by the enhancement of the tumour immunity, this in turn enhances the cell death of iC3b-opsonized tumour cells.

Not only the anti-tumour effect of the β-glucan but also their immunostimulatory effects was reported. With their effect on tumour immunity, the anti-tumour activity against colon cancer may be attributed to other factors such as induction of egestion of a carcinogen in the gut, regulation of the gut microbiota, and scavenging ROS. [7]

1.2. Applications of beta-glucans

Zymosan was arranged and examined out of the blue by Pillemer during the 1940s, and since that time it has been utilized in various examinations. Zymosan is a powerful trigger particularly of macrophages and it likewise actuates the arrival of a progression of cytokines from neutrophils. For a long time, it was not clear which part of this rough synthesis was in charge of its exercises. At the point when zymosan was inspected in detail, B-glucan was distinguished as the segment which had the essential impact. Glucan was in this manner confined and its immunological impacts were additionally examined.

Following the official of antibodies on the outside of malignancy cells, C3 pieces of supplement coat the disease cells. The β-glucan -prepared cells, for example, blood neutrophils, macrophages, and NK cells, at that point explicitly perceive these supplement immunizer edifices and slaughter the tumor cells. β-glucan participation with antibodies is along these lines a most encouraging blend treatment. [8]

In this paper, after comparing various methods for the extraction of β–glucan it has been observed that β–glucan best extracted from acidic extraction method using Oats and Barley in great amount which is used for cleansing product formation in bead form so that the little amount product is sufficient for use or only two beads for full face cleansing purpose. And due to these ingredients it is a healthy product that would provide nutrition, lock moisture in skin and it has anti-aging properties due to β–glucan.

II.METHODS

2.1. Reagents Required

K_2HPO_4, KH_2PO_4, MgSO4, NH_4Cl, Calcium Lactate (Dornish starch), Sodium Alginate (Merck), NaClO, HCl. Sodium Acetate, Perchloric acid, NaOH

2.2.Culture Required

Culturing of Yeast

Figure 2.1- Culture of Yeast

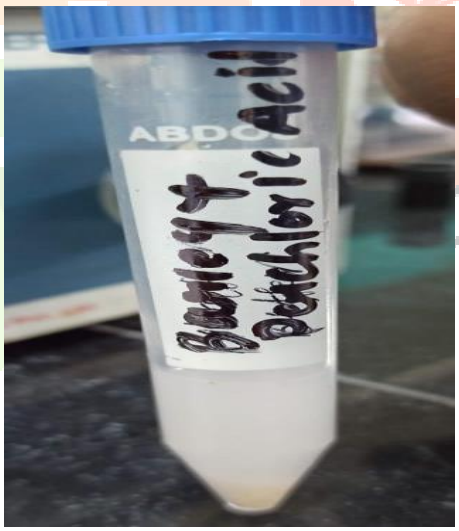

Figure 2.2- Barley Solution

2.3.Procedure

We selected cereals and try to make the process cost effective and less time consuming. Making the process more cost effective we use less ingredients with easy availability. We tried to change some steps or ingredients from the original source. We indeed were able to make some changes to got our results. Let's have look ahead.

2.3.1. Acidic Extraction of β- Glucan from Barley

1. Take 20 mg barley sample
2. Incubated for 5 min at 96℃ approx. with 50mM perchloric acid
3. Cooled the sample to room temperature
4. Then centrifuged the sample at 5000 rpm for 10 min
5. Remove the supernatant(supernatant1), and washed the pellet with perchloric acid
6. Again centrifuged the sample

7. Separate the supernatant(supernatant 2) and pellet
8. Now combined the supernatant 1&2
9. Washed it with distilled water and dry

2.3.2. Alkaline Extraction of β- Glucan from Wheat Bran

1. Take the pre-processed wheat bran and dissolve it in 1M NaOH for 2hr at 25℃
2. Then neutralize the sample with 2M HCI
3. After that centrifuged at 5000 rpm at 25 °C for 20 min.
4. Collect the supernatant and adjust the pH at 4.75 with 0.25M sod. Acetate buffer
5. Heat the supernatant at high temperature for deactivation
6. Centrifuged at 5000 rpm at 25 °C for 20 min.
7. Collect the supernatant and precipitate it with 50% Ethanol and thencentrifuged again
8. Washed the Ppt. with 50% ethanol
9. Suspend the Ppt. in 2-propanol at 4℃ overnight
10. Remove the solvent by drying the ppt. by warming
11. Wheat B-glucan

2.3.3. Extraction of Yeast β- Glucan

1. Preparation of Yeast Extract Glucose Broth (YG Broth)
2. Inoculate the S. cerevisiae strain in YG Broth and incubate the sample at 30℃ for 48 hr at 2000 rpm
3. Centrifuged at 7500 rpm for 10 min at 4℃ recover the yeast cell from the sample
4. Mixed the yeast cell with 5-fold of NaOH(1M)
5. Incubate the culture at 80℃ for 2 hr
6. Centrifuged the culture at 6000 rpm for 25 min at 4℃
7. Recover pellet and mixed it with 3-fold distilled water
8. Centrifuged the culture at 6000 rpm for 25 min at 4℃
9. Dissolved the pellet in 5-fold of HCl(1M) and incubate the culture at 80℃ for 2 hr
10. Centrifuged the pellet at 6000 rpm for 25 min at 4℃
11. Washed the pellet with distilled water 3 times
12. Dried the pellet in hot air oven at 60℃ and store the glucan pellet.

2.3.4. Alkaline Extraction of β- Glucan from Oats and Barley

1. Take barley/oat bran flour and dissolve in 1 N NaOH
2. Extracted at room temperature for 1hr
3. Centrifuged the sample for 15 min at 6000 rpm.
4. Collect the supernatant (supernatant1) and residue and re-extracted the residue with NaOH.
5. Again centrifuged the sample at 6000 rpm for 15 min.
6. Collect the supernatant (supernatant 2) and mixed supernatant (1)+(2)
7. Maintained pH 6.5+PDA
8. Incubated with shaking at 96 °C for 1 hr.
9. Cooled to 25 °C.
10. Centrifuged the sample at 6000 rpm for 15 min.
11. Collect the supernatant and washed it with 50% ethanol.
12. At room temperature, leave the sample overnight.
13. Centrifuged the sample at 6000 rpm for 15 min.
14. Crude gum suspended in water.
15. Washed twice with 50% ethanol.
16. Centrifuged again and lightly homogenized the pellets in water.
17. Collect the pellet and freeze dry.

2.3.5. Extraction of Omega 3

1. Flaxseed in powder form (10%w/v)
2. Incubate for 48 hour at -20 degree centigrade
3. Extraction with hexane
4. Centrifuge for 10 minutes at 2000 rpm
5. Removal of supernatant

6. Fractionation of supernatant with ethanol
7. Refined oil (Omega 3) extracted

I.3.6. Formation of Final Product

1. Add 1 g of sodium alginate to 1 cup of water.(Approx. 240 ml)
2. Then use an immersion blender to dissolve the sodium alginate and let the bubbles settle down.
3. Add 5 g of calcium lactate to 4 cups of water and mix well the solution.
4. Add 10ml of prepared ***cleansing solution** into calcium lactate mixture.
5. Scoop up sodium alginate solution using a deep spoon.
6. Plop the sodium alginate into the calcium lactate bath very carefully.
7. Make more beads with the remaining sodium alginate solution but do not crowd the bath.
8. Swirl the sodium alginate bubbles very gently.
9. After few minutes small- small transparent beads will be seen, which can be remove easily from calcium lactate mixture.
10. Collect the beads.

*** cleansing solution-** β–glucan, Omega-3, Vitamin C (Major Ingredients).

III.RESULTS

Figure 3.1: Depicts wheat bran pellets

Figure 3.2: Depicts extracted β-glucan

Figure 3.3 Depicts cleansing beads (β-glucan, omega3 and vitaminC)

Figure 3.4: Sample Beads

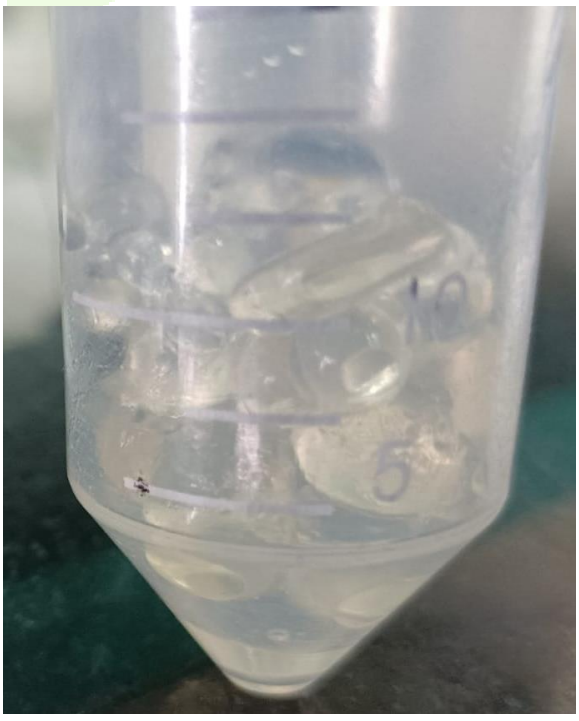

Figure 3.5 –Final Product

After comparing various methods for the extraction of β–glucan it has been observed that β–glucan best extracted from acidic extraction method using Oats and Barley in great amount which is used for cleansing product formation in bead form so that the little amount product is sufficient for use or only two beads for full face cleansing purpose. And due to these ingredients it is a healthy product that would provide nutrition, lock moisture in skin and it has anti-aging properties due to β–glucan.

IV.Discussion

β-glucan extracted from different sources has its own importance in different aspects. The concentration of extracted β–glucan varies according to the sources. β–glucan extracted from oats has higher concentration in comparison to other sources. The concentration of β-glucan also varies with different extraction methods. The optical density measured at 510 nm of oat β-glucan is 0.071 whereas of barley beta glucan is 0.063. Also the concentration of yeast β-glucan is 0.062. From observations we can conclude that oat has higher concentration of beta glucan. We can also conclude from market survey research on beta glucan that the demand is increasing not only in food and beverages sector but also in other sectors such as personal care, pharmaceutical, animal feed etc. And in coming years beta glucan will acquired much more market in India as well as globally.

Skin products have already acquired the market globally but most of the products are chemically composed. Nowadays public is demanding healthy skin care products. So the trends are shifting to naturally made products. β–glucan is extracted naturally from cereals; yeast etc. so it does not harm the skin and generally suitable for all skin types. So it's a good alternative to be used in combination with other natural resources for healthy skin care products. pH of the product should be maintained between 8-9 and store well to meet the all requirements. Further study is needed in this area.

The noteworthy observations noted from the discussion on various extraction methods are:-

i. Content of β-glucan differs with the nature of grain source and type of cultivar.
ii. Various types cultivars of the particular cereal grain may be having different levels of soluble and insoluble β-glucan
iii. Physicochemical properties of β-glucan differs, even from same cultivars, under different conditions while extracting.
iv. The role of various parameters namely, pH, temperature, extraction time, centrifugal gravity and purification, and isolation methods, is significant in the process of extraction.
v. Particle size of 0.5 mm gave much higher yield, while, on the other hand a decrease in the yield was notedusing finer flour.
vi. Before the procedure of extraction, for the prevention of degradation of beta-glucan it is advised to deactivate the innate β-glucanase enzyme with ethanol treatment.
vii. When the temperature is set at 80°C than at ambient temperature deactivation of innate β-glucanase enzyme is faster.
viii. While non-solubilized starch removal requires extraction below 50 °C, solubilized starch in extract may be removed by treatment with the specific enzyme.
ix. Removal of protein can be done by precipitation at 4.5 pH with (NH4)2SO4 or with the specific enzyme.
x. Purification of β-glucan may be done either by repeated centrifugation of the extract followed by freeze-drying of the supernatant or by precipitation with alcohol followed by vacuum drying the precipitate containing β-glucan.
xi. β-glucan precipitated with alcohol is not easily dissolvable in water that affects its targeted biological action.
xii. The higher is the temperature while the process of extraction is taking place the high MW β-glucan is obtained. But temperature as high as 160° C or more degrades the β-glucan.

xiii. When the process of extraction is done at high pH degradation of β-glucan occurs. Thus, lowering the MW.

xiv. Low MW β-glucan means low viscosity and low-viscosity β-glucan is not that much effective in comparison with the higher viscosity. Also, low viscosity β-glucan has poor gelling property which is considered bad when talking through the industrial point of view.

xv. The total recuperated yield of β-glucan from barley bran was higher than that from oat grain while virtue of β-glucan removed from oat was higher.

xvi. The MW of β-glucan separated relied on sort of cultivar and extraction conditions and observed to be more prominent than 2.0×106 Da for every one of the cultivars talked about above.

xvii. Variety in the viscosities of β-glucan arrangements saw amid consistency judgments was ascribed to contrasts in molecular size and fine structure.

xviii. The other method used by Beer and others gave different results. They used the same alkaline method but with some simple changes. And these simple changes made some great differences in the yield, recovery and kinematic viscosity of beta-glucan.

xix. Effect of enzyme deactivation on β-glucan content and kinematic viscosity of the extracted gum was studied by comparing the β-glucan isolated from untreated flour and it was observed that the yield from untreated flour was higher though β-glucan content and viscosity were lower. Oat that have been deactivated by enzyme gave lower yield but with higher purity and viscosity that was in accordance with reports by Wood and others (1989). Dialysis produced the gum of higher viscosity in comparison to ultrafiltration and alcoholic precipitation. β-glucan isolated by dialysis and ultrafiltration was found to dissolve in water rapidly while that precipitated with alcohol was not easily dissolvable in water.[26]

xx. Be very careful while scooping the beads, otherwise they may get damaged

xxi. Mixing of sodium alginate is done well and make sure before using the solution bubbles get settled down.

V.CONCLUSION

The extraction of β-glucan using various methods like Acidic Extraction, Alkaline Extraction of β- Glucan from Barley, Wheat Bran, etc and after comparing these various methods for the extraction of β–glucan it has been observed that β–glucan best extracted from acidic extraction method using Oats and Barley in great amount which is used for cleansing product formation in bead form so that the little amount product is sufficient for use or only two beads for full face cleansing purpose. And due to these ingredients it is a healthy product that would provide nutrition, lock moisture in skin and it has anti-aging properties due to β–glucan.

VI.Abbreviations

β-glucan: Beta glucan; YG: Yeast extract glucose, MW: Molecular weight; Da: Dalton; ROS: Reacting oxygen species.

VII.SUMMARY

With advancement in technology, the number of health problems which were considered uncurable are now being cured. But the change in lifestyle nowadays compared to the past times, there are a lot of new health problems generating. And, due to the lack of resources, we need something effective with multiple qualities. β- glucans are a class of polysaccharide. The purpose of this study is to investigate all the functions of β-glucans which can be helpful. β- glucans were chosen because they are present in various food products like mushrooms, cereals and seaweeds. They are known to have a diverse range of structures which is the reason for their diverse functions. They effect the human body in plentiful ways. They help in the prevention of absorption of cholesterol. They also have a lot of skin benefits. They show anti- oxidant properties, immunostimulatory, anti-ageing effects, wound healing and antitumor effects also. They improve the intestinal microbiota. In this report, various methods of extracting β-glucan from different sources had done in our lab then select the best method for this production. Extraction of β- glucans was done through Oats & Barley to create a healthy nutritive cosmetic product with highly nutritive other ingredients.

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