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Formulation & Evaluation of Margarine with Single-Cell Oil of Sesamum indicum, Helianthus annuus, Linum usitatissimum & Glycine max

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ABSTRACT

Margarine is a good choice as it is cost-effective, adaptable, and an ideal substitute for butter. Butter has high content of saturated fatty acids that can increase the risk of cardiovascular and other heart related diseases. Margarine is oil based and its nutritional values depend on precise relationship between oil blends' compositions and their elements. The margarine was formulated using oil blends of Sesamum indicum, Helianthus annuus, Linum usitatissimum and Glycine max. This research utilized four vegetable oils, Sesamum indicum, Helianthus annuus, Linum usitatissimum and Glycine max attributed to its nutritional value. The oil blends were fermented using isolated yeast strain to form single cell oil which was further used in the formulation of margarine. The formulated margarine was assessed on sensory analytical and nutritional parameters. The results showed the required carbohydrate and protein contents in the samples were 0.1584 mg/ml, 0.1479 mg/ml & 0.0403mg/ml respectively for carbohydrate and 0.0103 mg/ml, 0.0304 mg/ml & 0.0261 mg/ml respectively for protein. Based on the above observations, margarine can be suggestive as an affordable and healthy option as compared to dairy butter having lower content of saturated fats and a margin to be fortified by adding vitamins A and D to enhance its nutritional potential.

KEYWORDS: *Single-cell oil, margarine, Glycine max, Helianthus annuus, Linum usitatissimum and Sesamum indicum*

1. INTRODUCTION

Butter is considered as an integral part of the Western civilization. In ancient Rome, it was employed as a medication, and it was consumed for coughs or rubbed to hurting joints. For almost 3000 years, Hindus have offered it to their gods as ghee which is a clarified butter. In the Bible, butter has been mentioned as a celebratory dish. Butter was handmade until the nineteenth century. [1] Vitamins A, D, E, Calcium, and conjugated linoleic acid are abundant in butter. Butter consumption in moderation has been related to lower obesity, diabetes, and heart disease risks. [2] The manufacture of margarine that consists of high polyunsaturated fat can compensate a healthy diet. It's high in minerals including calcium, which aids in bone formation and protection, and it has chemicals linked to reduced risk of obesity. Butter, regardless of grade, have a minimum fat level of 80 percent, which is far too high for many dietary related

disorders and also for diet conscious people. Resultantly, wide range of low-fat spreads have been developed and marketed. Spreads can be produced with vegetable oils and butter fats.

Hippolyte Mege Mouries, a French chemist created margarine to aid butter shortages raised during Industrial Revolution and World War II in 1869. [4]-[7] Slowly margarines were made using unfractionated cow suet and cottonseed or peanut oils. Vegetable oil margarines with coconut and palm kernel oils were produced in the 1900s. [3] It's known for being both a nutritious and cost-effective table spread. Margarine is an emulsion of water and oil with several classifications depending on its components. [4] As people's health awareness and consumption levels are rising, they're paying greater attention to healthier plant oil-based margarines. The broad popularity of margarine is largely due to enhanced "tailor-made" commodities to fulfil consumer perceived convenience. Aside from introduction of new oils, significant improvements to the manufacturing process were achieved. The process of Pasteurization made it possible to cultivate milk, in bulk amounts. Dry chilling, involving circulation brine in a metal drum, had lowered fat involved and milk content hygienically. The evolution of margarine influenced by legislation called for government intervention due to use of imported tropical oils in the 1960s. In 1886, the first tax on colored margarine was imposed, followed by a considerably harsher tax and producer licensing costs in 1902. Margarine has numerous purposes, such as cooking and spreading on bread and other dishes. Earlier, it was available as a rigid stick, because of constant fat content present in it. In present times, it is available in varied forms as fluid, squeezable product, and also in soft tub form, labelled as margarine or diet margarine depending on the fat ratio.

Single Cell Oil (SCO), also known as microbial oil, are lipids produced by oleaginous microorganisms because to their distinct characteristics. Due to its dual function as a source of functional oil and a feedstock for biodiesel production, it received a lot of attention. Oleaginous microbial species have 20% of their dry weight as lipids. [5]–[10] The research on SCO revealed that it might be helpful for human health being an excellent substitute to expensive materials like cocoa butter. Low DHA levels, as well as polysaturated fatty acids (PUFA), omega-3 fatty acids (FA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) found in SCO, have been linked to number of health benefits, including reduced risk of vision loss, reduced blood vessel stiffness, relief from anxiety and inflammation, and treatment of diseases such as asthma, arthritic, depression, and migraines. Greater part of these lipids has triacylglycerols (TAG) and steryl esters (STEs), which are crucial for storage of lipids in eukaryotic cells. The most extensively used oil-producing species are eukaryotes. Related research is still in juvenile phase, with promising results from fish and plant-based sources. As waste substrates with zero or negative value are taken as carbon and nitrogen sources for fermentation, SCO's economic power continues to ameliorate. [11]–[17] The oil blend composition, yeast strains, growth conditions, and medium all contribute towards the efficiency of SCO. Due to the ongoing global energy crisis, SCO appears to be a great choice in fields such as medicine, pharma, food, and biodiesel production, among others. Microbial lipids might also be used as antimicrobial agents in cosmetics, additional diets, and infant formulas. [18]

Sesamum indicum (S. indicum)

This oil is used by Indians since early ages, and many traditional winter foods in India. Because of its health benefits, *S. indicum* oil is known as the "Queen of Oil seeds." It is high in antioxidants, which aid in reducing cell damage and inflammation. It contains high amounts of omega-6 unsaturated fatty acid, which helps in prevention of heart diseases to certain levels. It also assists in the regulation of blood sugar, treatment of arthritis, protection against UV rays, helpful in insomnia, pain relief, and improvement of hair and skin health. [19]

Helianthus annuus (H. annuus)

Helianthus annuus is commonly known as Sun flower. According to some archaeologists, sunflower may have been cultivated before rice. This plant has been cultivated by humans approximately from 3000 BC. Sunflowers are utilized in varied ways since early. Ground or pounded seed are used to make cakes, mush, and bread. Vitamin E content in *H. annuus* oil is high with high oleic content, which lowers LDL (low-density lipoprotein) or bad cholesterol levels while raising HDL (high-density lipoprotein) or good cholesterol levels which in turn lowers the coronary heart disease risk. It also contains

vitamins B1, E, and K, which have favorable effects on brain and nerve health. It also helps in boosting energy levels. [20]

Linum usitatissimum (L. usitatissimum)

Linum usitatissimum oil, commonly known as Linseed oil/ flaxseed oil, is extracted from the flax plant's dried, ripened seeds. As it is extracted from dry plant solids, it can polymerize and solidify. This is also is well-known for its high levels of heart-healthy omega-3 fatty acids, protein, and fiber. It aids digestion and reduces the chances of lifestyle diseases as cardiac diseases and diabetes. Antioxidants in *L. usitatissimum* oil majorly comprises of p-Coumaric acid and Ferulic acid.

Glycine max (G. max)

G. max oil is one amongst the majorly consumed dietary oils. It has become popular due to both of its nutritional and economic perks. [21] It is rich in polysaturated fats which help in lowering of LDL levels and thus reducing the risk of atherosclerotic disease. It also supports bone health attributed to its Vitamin K content. Omega-3 fatty acids and Vitamin E are also reported to be helpful in prevention of chronic diseases and promoting good skin health. [22]

Saccharomyces cerevisiae (S. cerevisiae)

Saccharomyces cerevisiae popularly known as nutritional yeast or baker's yeast, is widely used in varieties of cuisine around the world. It is low in sodium, calories, fat-free, gluten free, and vegan by nature. It is high in its nutritional content specifically in protein, vitamin B and trace minerals. It also helps vitamin B deficiency. It has antioxidants such as glutathione and selenomethionine, which aid in the removal of environmental toxins by the body providing defense against chronic illness. [23] Although, there are only a few evidences available in relation to moving to a low-fat, low-saturated-fat diet reducing the risk of lifestyle diseases or mortality. Despite this, attention is increasingly being drawn towards nutritional supplements and metabolites in meals. Hence developing the concept of "fortified and nutritive meals" is on rise around the world.

2. Materials and Methodology

A. Pure culture isolation

Food grade Saccharomyces cerevisiae was revived and prepared to be used as starter culture for the fermentation of Single Cell Oil (SCO). S. cerevisiae isolation was carried out on YEPD medium. The isolated cultures were stored in YEPD broth and centrifuged to obtain the cell pellet when required for further use.

B. Preparation of Margarine

Single Cell Oil (SCO) was formulated by the fermentation of different oils blends. These oil blends were fermented with the use of S. cerevisiae. The cell pellet was dissolved in 10 ml of an oil blend and was allowed to ferment for 72-96 hours for the formation of SCO. This was followed by emulsification. The formulation was further optimized by addition of Starch, antioxidant (Vitamin E) and food color (Yellow food dye) to form SCO mix. This concoction was heated at 50ºC on a water bath and a water-based solution of sodium chloride, flavor (vanilla) and milk solids were added in different concentrations under continuous stirring followed by SCO mix. The whole solution was cooled in 10% sodium chloride ice bath and allowed to cool till a thick consistency paste was obtained. Adjustments were done further for improvement in flavor and texture. These semi-liquid batches were kept in -20ºC to solidify for 24 hours resulting in the formation of margarine, which was further stored at +4ºC until further evaluation. [24]

3. Evaluation of Margarine

Sensory and nutritional evaluation was carried out for all the prepared batches. The product was evaluated and finalized on the basis of major acceptability by volunteers on varied parameters as taste, appearance, texture, mouth feel and other sensory parameters. The shortlisted formulations were further subjected to nutritional assessments.

A.Sensory Evaluation

The analysis was derived on the 9-point Hedonic Scale. It is widely used for measuring food and beverage acceptability, personal health care products, household products and cosmetics. Sensory analysis was carried out as per the standard questionnaire by 30 volunteers, and results were documented for further analysis.

B. Nutritional evaluation

a) Carbohydrate Estimation Test

Carbohydrate content was estimated by classic DNS method. The margarine samples were diluted with Distill water into different dilutions for carbohydrate estimation. Absorbance was noted at 540 nm. The results were plotted against standard curve for dextrose for the calculation of carbohydrates present.

b) Protein Estimation Test

Protein estimation was carried out by Folin method. Bovine Serum Albumin (BSA) (stock - 1mg/ml) was used as standard. The absorbance was measured at 660 nm. The results were then plotted against BSA standard curve to find the protein content in the formulated product.

4. Results

The formulated margarine batches were subjected to sensory analysis, followed by nutritional assessment. Figure 1 shows the formulated margarine batches accepted after sensory evaluation. Figure 2 shows the standard curve for dextrose used to find the concentration of carbohydrate in formulated margarine batches. Table 1 shows the sensory analysis of the margarine samples B1, B2 and B3. Table 2 shows the concentration of required carbohydrate content calculated from the graph i.e., B1= 0.1584 mg/ml, B2= 0.1479 mg/ml & B3= 0.0403 mg/ml respectively. Similarly, Table 3 shows the protein analysis determined of required protein content in the samples i.e., B1= 0.0103 mg/ml, B2= 0.0304 mg/ml & B3= 0.0261 mg/ml respectively. Figure 3 shows the standard absorbance curve and concentration of protein in unknown samples B1, B2 and B3.

5. Figures and Tables

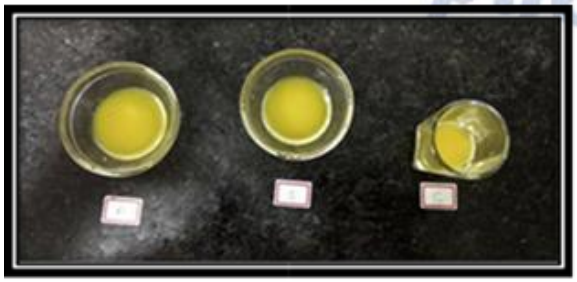

Figure 1: Final formulated margarine batches

Criteria	Batch A	Batch B	Batch C
Taste	6.1	7.1	6.7
Texture	7.2	7.7	6.9
Aroma	6.9	7.2	7.8
Appearance	7.6	7.2	7.7
Colour	8.3	7.6	7.0
Mouthfeel	6.3	7.1	6.3
Overall Acceptability	7.4	7.6	7.2

Table 1: 9-scale Hedonic sensory analysis

Batch	Carbohydrate Concentration (mg/ml)
B1	0.1584
B2	0.1479
B3	0.0403

Table 2: Carbohydrate evaluation

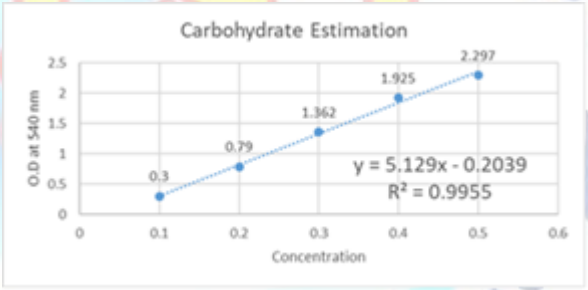

Figure 2: A plot of a standard curve of absorbance of carbohydrates and concentration of carbohydrates in unknown sample extracts

Batches	Protein Concentration (mg/ml)
B1	0.0103
B2	0.0304
B3	0.0261

Table 3. Protein evaluation

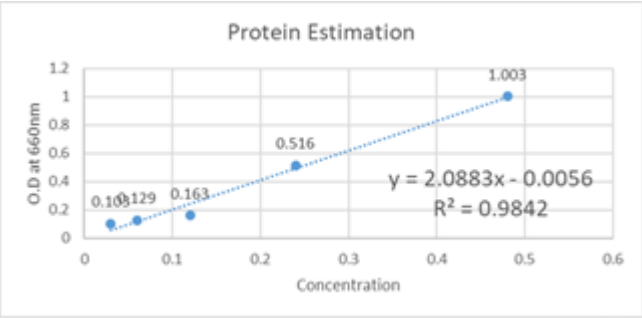

Figure 3: A plot of a standard curve of absorbance of protein and concentration of protein in unknown sample extracts

6. Conclusions

Lastly, the sensory evaluation survey was conducted on 30 people based on which the data of table 5 was obtained. In middle and low-income households, 85% deaths happen due to heart stroke and heart, whereas CVDs account for over 75%. This research backs up the hypothesis that trans-poly unsaturated fatty acids, when ingested in moderation, have no harmful effects on the human body. Plant oils have a longer chain of trans PUFAs, which are heart-healthy, as opposed to animal fats, which have a higher concentration of SFAs and HDL, which can lead to heart disease. To add to the benefits, SCO includes a high level of natural anti-oxidant, which protects omega 3 fatty acids from oxidation and allowing them to last longer, lowering blood pressure and slowing the ageing process. The margarine used in this study was made with high-nutrition vegetable oils. The margarine's nutritional analysis revealed that the amount of carbs and protein present in the sample compared to the entire amount of margarine produced was in compliance with Food Safety and Standards Authority of India norms.

However, according to Harvard Medical School, margarine and butter should only be used on occasion, and healthier alternatives such as olive oil and other vegetable oil-based spreads should be chosen over margarine due to the number of calories and trans fats contained. On the basis of taste, texture, smell, and mouth feel, a sensory study of margarine created found that the product was satisfactorily accepted by the participants. Although margarine is commonly thought to be a healthier substitute to butter, there is some evidence suggesting the contrary. Hence, there is a need for the development of margarine by newer methods like formation from SCO, which can add up few more beneficial properties to the product formed and lower the amount of trans fat in it making it a healthy choice. In conclusion, People who follow a vegan diet and make health-conscious food selections are more likely to use margarine. It's a rancidity-free water-in-oil emulsion that's free of mineral oils and animal fats. It is quite light on the body and may be tailored to suit individual needs using a variety of vegetable oils available on the market. Because of the dramatic increase in margarine use over the last decade, which may be attributed to economic factors as well as medical advice and commercial advertising, a comparison of margarine's nutritional value to butter or oils in terms of preventing atherosclerosis is necessary. Modern margarine is a highly processed food manufactured from vegetable oils that can be easily made with SCO and yeast.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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