Optimization of cocoa butter analog synthesis variables using neural networks and genetic algorithm

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Abstract Cocoa butter analog was prepared from camel hump fat and tristearin by enzymatic interesterification in supercritical carbon dioxide (SC-CO₂) using immobilized *Thermomyces lanuginosus* lipase (Lipozyme TL IM) as a biocatalyst. Optimal process conditions were determined using neural networks and genetic algorithm optimization. Response surfaces methodology was used to design the experiments to collect data for the neural network modelling. A general regression neural network model was developed to predict the response of triacylglycerol (TAG) distribution of cocoa butter analog from the process pressure, temperature, tristearin/camel hump fat ratio, water content, and incubation time. A genetic algorithm was used to search for a combination of the process variables for production of most similar cocoa butter analog to the corresponding cocoa butter. The combinations of the process variables during genetic algorithm optimization were evaluated using the neural network model. The pressure of 10 MPa; temperature of 40 °C; SSS/CHF ratio of 0.6:1; water content of 13 % (w/w); and incubation time of 4.5 h were found to be the optimum conditions to achieve the most similar cocoa butter analog to the corresponding cocoa butter.

Keywords Enzymatic interesterification · Camel hump fat · Supercritical carbon dioxide (SC-CO₂) · Neural networks · Genetic algorithm

Introduction

Cocoa butter, which amounts to 25–36 % in finished chocolate, is responsible for the smooth texture, contractibility, flavor release, and gloss of the product (Liu et al. 1997). Cocoa butter is composed predominantly (>70 %) of three symmetrical TAGs, 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1(3)-palmitoyl-3(1)stearoyl-2-oleoylglycerol (POS), and 1,3-distearoyl-2-oleoylglycerol (SOS). Numerous factors, however, including the degree of uncertainty in supply, variability in quality, and price premium compared to other fats have driven oil industry for alternatives (Hartmann et al. 2001). Producing fats with highly desirable physicochemical properties, such as cocoa butter alternatives by enzymatic interesterification in organic solvents, has become a popular area of biotechnological research (Mojovic et al. 1993; Silroy et al. 2011). However, organic solvent systems are suffering from some major drawbacks such as final solvent removal, which is usually costly and time and energy consuming. The use of supercritical fluid as a solvent and reaction medium for lipids has grown rapidly in recent years. In addition, the solubility of most organics in supercritical fluids is higher than that in gaseous phase and is comparable with liquid solvents. Among supercritical fluids, SC-CO₂ is especially advantageous due to low toxicity, viscosity, cost, surface tension (Kumar et al. 2011). The solvent properties of supercritical carbon dioxide can be readily modified by adjusting pressure or temperature; the diffusivity of solutes in carbon dioxide is higher than in organic solvents. Carbon dioxide can easily be removed from the reaction products to minimize the need for costly downstream clean-up. When carbon dioxide is used in lieu of organic solvents, it has the additional benefit of being an environmentally benign process (Jackson and King 1997; Liu et al. 2007).

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Camel is unique in having an exceptional ability to survive and thrive under adverse climatic conditions such as high ambient temperatures, low rainfall, and feed scarcity. The camel stores its energy reserves in the form of fat in different depots in their body of which the hump comprises a considerable amount of the adult body weight. Hump usually contains 70–90 % fat, the balance being water and connective tissue. Hump fat contains mixtures of fatty acids and most of them are C16:0, C18:0 and C18:1 (Kadim et al. 2002).

Neural network is a computational technique which mimics the structure and function of human brain to some extent (Ruhil et al. 2011). Neural networks can be used to develop an approximation function or a relationship between the inputs and outputs of the system. Once a neural network model is developed, the model can be used in a genetic algorithm optimization algorithm to evaluate the performance of the combinations of control variables (Moghaddam 2010).

In supervised learning algorithms, neural networks are trained and an approximation function or a model is developed. During the training, the inputs and outputs of the system are cycled through the network repeatedly to adjust the weights between the neurons in the network layers. The trained network then can be used to predict the outputs for newly-introduced inputs (Koc et al. 2007).

Genetic algorithms (GAs) are powerful optimization techniques based on the methods of evolution. Genetic algorithm optimization includes the generation of possible solutions, application of selection, crossover and mutation operations and evaluation of each solution over an objective function (fitness function) until a certain stopping criterion is met (Rivera et al. 2006).

In the present work, it was found that cocoa butter analog could be efficiently synthesized by interesterification with immobilized *Thermomyces lanuginosus* lipase (Lipozyme TL IM) as biocatalyst in SC-CO₂. A general regression neural network (GRNN) was applied to fit the relationship between the condition parameters including pressure, temperature, tristearin/camel hump fat ratio, water content, and incubation time and responses including POP, POS and SOS content of cocoa butter analog. GA-based optimizer was developed for constrained nonlinear programs involving expressions obtained from GRNN.

Materials and methods

Materials

Immobilized *Thermomyces lanuginosus* 1, 3-specific lipase (Lipozyme TL IM) with the enzyme activity of more than 140 IU/g was the gift provided by Novozymes (Tehran, Iran). Fatty acids and tristearin were purchased from Sigma (St. Louis, USA). Cocoa butter was purchased from Cocoa

PI pressure indicator.

Fig. 1 Schematic diagram of supercritical carbon dioxide system for enzymatic reaction

Tabriz Co. (Tabriz, Iran). Camel hump fat was extracted by batch dry rendering from camel hump which was obtained from slaughterhouse. Chopped camel hump was heated at 90 °C for 5 h. Extracted oil was separated from camel hump tissue by filtration. Tetrahydrofuran and isopropanol used for HPLC analysis were spectral grade and obtained from Merck. The purity of CO₂ was more than 99.95 % and was purchased from ZamZam Co. (Isfahan, Iran). Other chemicals and solvents were all analytical grades that were purchased from Merck.

Enzymatic interesterification

The experimental apparatus with SC-CO₂ as the reaction medium is schematically shown in Fig. 1. Cocoa butter analog synthesis was carried out in a SC-CO₂ batch reactor, a 40 mL-high pressure vessel into which camel hump fat (CHF) and tristearin (SSS) were blended at various weight percent ratios. Then, immobilized lipase (10 % by total weight of substrate) and water at various weight percents of substrate were added. Reactions were carried out under different conditions of reaction pressure, temperature, and incubation time. Design of experiments through a central composite design (CCD) technique via a response surface method (RSM) using Minitab 14 environment determined the number of experiments to be conducted (Rafieian et al. 2011). The experimental design included 32 experiments of five variables at five levels with six replications. Table 1

Table 1 Experimental range and values of the independent process variables in the design for production of cocoa butter analog

Independent variables	Levels				
Reaction pressure(MPa)	3	10	17	24	31
Reaction temperature(°C)	30	40	50	60	70
Reaction time (h)	1	2.5	4	5.5	7
Water (%)	0	5	10	15	20
SSS/CHF Ratio	0.125:1	0.25:1	0.5:1	1:1	2:1

SSS/CHF tristearin/camel hump fat.

gives the range of variables employed. After reaction to the desired time, depressurization and elution by n-hexane was conducted. The reaction mixture was filtered immediately through a 0.45 µm filter to remove the enzyme. Hexane was removed by rotary vacuum evaporator.

RP-HPLC analysis

HPLC was carried out with a Hewlett-Packard model 1090 high-performance liquid chromatographer equipped with an auto sampler, quaternary pump module, and a UV/VIS scanning (diode array) detector. The TAG mixtures were separated by a non aqueous spherical RP-C18 column (150 mm×3.9 mm×5 µm). Separations were carried out with acetonitrile/tetrahydrofuran (75:25, v/v as solvent A) and water (as solvent B) as eluents at a flow rate of 1 mL/min and the following solvent gradient profile: initial condition 60:40 (volume percent A/B), to 100 % A over 10 min, held for 15 min, and then returned to original conditions over 5 min (the total run time was 30 min). The UV spectra of TAG were determined at 220 nm. The oven temperature was set at 40 °C. The samples were prepared at 25 mg/mL in tetrahydrofuran and 20 µL of the solution was injected. The peak identification was done by comparing the retention time with those of TAG standards (Sridhar et al. 1991).

Neural networks (NN) analysis

NNs technique is a data processing tool that mimics the function of the human brain and nerves built on the so-called neurons –processing elements– connected to each other. This technique differs from traditional data processing; it learns the relationship between the input and output data (Ciftci et al. 2008).

GRNN is a type of supervised network, and has been generally accepted for its excellent capability to train

rapidly on sparse data sets. The GRNN has four layers. The number of neurons in the first layer (input layer) is equal to the number of inputs and the number of neurons in the fourth layer is equal to the number of outputs (output layer). The number of neurons in the second layer (pattern layer) and the third layer (summation layer) is equal to or more than the number of training data sets. The data is scaled using a scaling function before it is used for training. During the training of GRNN, a neuron for each training pattern is assigned in the pattern layer. The weights between the pattern layer neurons and the input layer neurons are equal to the inputs of the training patterns. The outputs of the training patterns are assigned as the weights between the pattern layer neurons and the summation layer neurons. Figure 2 presents the GRNN block diagram. It can estimate values of Y (target variables) for any new value of X (input variables) through the multi-layer network. From the Parzen's nonparametric probability density estimate, the fundamental formulation of the GRNN is deduced as follows:

$$\hat{Y}(X) = \frac{\sum_{i=1}^n Y_i \exp \left[-\frac{(X-X_i)^T (X-X_i)}{2\sigma^2} \right]}{\sum_{i=1}^n \exp \left[-\frac{(X-X_i)^T (X-X_i)}{2\sigma^2} \right]}$$

Where X_i is the vector of input parameters, Y_i is the vector of response of the experiment associated to the input vector (X_i), $\hat{Y}(X)$ is the estimate associated with any value of input vector (X), n is the number of experiments and σ is the smoothing parameter, i.e. the kernel width of the Gaussian function (Specht 1991).

A multilayer GRNN was designed in the MATLAB environment employing the data obtained from the experiments conducted.

Fig. 2 Representation of a GRNN

GRNN general regression neural network, POP 1,3-dipalmitoyl-2-oleoylglycerol, POS 1(3)-palmitoyl-3(1)-stearoyl-2-oleoylglycerol, SOS 1,3-distearoyl-2-oleoylglycerol, SSS/CHF tristearin/camel hump fat.

Genetic algorithms

Genetic algorithms (GA) have been used widely for dealing with optimization problems. GA is used to optimize a given objective function over a given search space.

A population of individuals undergoes some artificial Darwinian evolution (genetic inheritance and Darwinian strife for survival) based on the fitness of each individual. The fitness of an individual is directly related to the value of the objective function of this individual. The manipulation is done by the genetic operators that work on the chromosomes,

Table 2 Responses of dependent variables for production of cocoa butter analog to independent variables

Exp. No.	Variable levels					TAG (%)		
	X1	X2	X3	X4	X5	POP	POS	SOS
1	17	50	4	10	0.5	24.98	41.18	33.84
2	17	50	4	10	0.5	26.38	40.83	32.78
3	24	40	5.5	5	1	21.28	41.4	37.32
4	24	50	2.5	15	0.25	26.83	37.23	35.94
5	10	40	5.5	5	0.25	17.68	38.68	43.63
6	3	50	4	10	0.5	20.62	41.27	38.11
7	17	30	4	10	0.5	24.4	40.41	35.19
8	17	50	4	10	0.5	25.55	43.28	31.16
9	24	40	2.5	15	1	19.72	44.53	35.75
10	10	60	5.5	5	1	27.38	38.56	34.05
11	10	60	5.5	15	0.25	17.26	38.7	44.03
12	24	60	5.5	5	0.25	36.7	37.58	25.71
13	17	50	4	10	0.125	25.99	39.39	34.62
14	24	40	2.5	5	0.25	25.15	36.32	38.53
15	10	40	5.5	15	1	24.56	43.64	31.79
16	31	50	4	10	0.5	24.56	40.46	34.97
17	17	50	4	0	0.5	30.72	38.67	30.6
18	17	50	4	10	0.5	27.8	39.34	32.87
19	17	50	4	10	0.5	25.99	40.34	33.67
20	24	60	5.5	15	1	32.41	37.26	30.32
21	24	40	5.5	15	0.5	19.4	43.74	36.85
22	10	50	4	10	2	34.08	42.35	23.56
23	17	70	4	10	0.5	27.64	37.08	35.27
24	17	50	7	10	0.5	29.09	39.93	30.98
25	17	50	4	20	0.5	28.88	42.85	28.27
26	17	50	1	10	0.5	28.68	38.16	33.16
27	10	40	2.5	15	0.25	21.05	40.42	38.52
28	10	60	2.5	5	0.25	37	38.88	24.11
29	17	50	4	10	0.5	27.65	40.74	31.59
30	10	40	2.5	5	1	21.88	42.26	35.85
31	24	60	2.5	5	1	29.26	37.85	32.88
32	10	60	2.5	15	1	22.61	37.04	40.34

TAG triacylglycerol, POP 1,3-dipalmitoyl-2-oleoylglycerol, POS 1(3)-palmitoyl-3(1)stearoyl-2-oleoylglycerol, SOS 1,3-distearoyl-2-oleoylglycerol

in which the parameters of possible solutions are encoded. In each generation of the GA, the new solutions replace the solutions in the population that are selected for deletion (Cravener and Roush 2001).

Here in this paper, to find the best content of POP, POS and SOS in the cocoa butter analog, the GA optimization method was adopted based on the GRNN model.

Results and discussions

The content of POP, POS, and SOS of cocoa butter analog obtained from all the experiments are listed in Table 2.

The character of the neural network requires that all variables (i.e. pressure, temperature, time, water and SSS/CHF ratio) influencing the model output (POP, POS and

Table 3 Estimated responses via GRNN

Exp. No	POP(%)	POS(%)	SOS(%)
1	26.36	40.78	32.86
2	26.36	40.78	32.86
3	21.28	41.40	37.32
4	26.83	37.23	35.94
5	17.68	38.68	43.63
6	20.62	41.27	38.11
7	24.40	40.41	35.19
8	26.36	40.78	32.86
9	19.72	44.53	35.75
10	27.38	38.56	34.05
11	17.26	38.70	44.03
12	36.70	37.58	25.71
13	26.32	40.67	33.01
14	25.15	36.32	38.53
15	24.56	43.64	31.79
16	24.56	40.46	34.97
17	30.72	38.67	30.60
18	26.36	40.78	32.86
19	26.36	40.78	32.86
20	32.41	37.26	30.32
21	19.40	43.74	36.85
22	33.57	42.26	24.16
23	27.64	37.08	35.27
24	29.09	39.93	30.98
25	28.88	42.85	28.27
26	28.68	38.16	33.16
27	21.05	40.42	38.52
28	37.00	38.88	24.11
29	26.36	40.78	32.86
30	21.88	42.26	35.85
31	29.26	37.85	32.88
32	22.61	37.04	40.34

GRNN general regression neural network, POP 1,3-dipalmitoyl-2-oleoylglycerol, POS 1(3)-palmitoyl-3(1)stearoyl-2-oleoylglycerol, SOS 1,3-distearoyl-2-oleoylglycerol.

Fig. 3 GRNN predictions (POP, POS and SOS) and experimental values for training sets

GRNN general regression neural network, POP 1,3-dipalmitoyl-2-oleoylglycerol, POS 1(3)-palmitoyl-3(1)stearoyl-2-oleoylglycerol, SOS 1,3-distearoyl-2-oleoylglycerol

SOS content) be included in the input layer to allow the finding of the relationships that exist in the data. Producing of training data is an important issue in determining the quality of estimate. In this study, the experimental conditions presented in Table 2 were taken as the training set. Each training pattern had the combination of various values of input condition parameters, and the related responses. The GRNN training was completed using the training data set. Best smoothing constant (σ) after training was determined to be 0.592 by using the genetic algorithm method.

The responses, POP, POS and SOS content of cocoa butter analog, were approximated into response surface using GRNN. Table 3 lists the estimated results approximated from

GRNN. After the training, the fitted responses surface was very close to the desired values. Figure 3 compares the NN predictions to the experimental data via scatter plots for training set. The overall performance of training set was evaluated by the determination coefficient (R^2) values ($R_{POP}^2 = 0.991$, $R_{POS}^2 = 0.932$ and $R_{SOS}^2 = 0.986$). It is clear that the proposed model had impressively learned the nonlinear relationship between the input and the output variables which exhibited a successful performance of the GRNNs model for prediction of POP, POS and SOS content of cocoa butter analog in the training stage.

The mathematical model for finding the content of POP, POS and SOS of cocoa butter analog can therefore be constructed by using the equations arising from the trained GRNN model.

Table 4 Comparison of experimental and predicted results

TAG (%)	Experimental value	Predicted value
POP	21.56	21.67
POS	41.44	41.80
SOS	36.99	36.51

TAG triacylglycerol, POP 1,3-dipalmitoyl-2-oleoylglycerol, POS 1(3)-palmitoyl-3(1)-stearoyl-2-oleoylglycerol, SOS 1,3-distearoyl-2-oleoylglycerol.

An experiment with arbitrary conditions (pressure=10 Mpa, temperature=40 °C, time=3 h, water=10 % and SSS/CHF Ratio=1:1) was conducted to verify GRNN and in this regard predicted values and experimental values of TAG content of cocoa butter analog were compared (Table 4). Results indicated that the experimental values are very close to those that were predicted. Therefore, the GRNN model can be adapted for optimization of cocoa butter analog processing from camel hump fat.

To determine the best input conditions while keeping the main TAG of cocoa butter analog most similar to the main TAG of natural cocoa butter (23.82 % POP, 44.81 % POS, and 31.37 % SOS), the GA optimization method was applied to minimize the below objective function (F):

$$F = (\text{POP} - 23.82)^2 + (\text{POS} - 44.81)^2 + (\text{SOS} - 31.37)^2$$

After 50 generations, the best input condition was obtained as follows: Pressure=10 Mpa, Temperature=40 °C, Time=4.5 h, Water=13 %, SSS/CHF Ratio=0.6:1. By applying these parameters, GRNN predicted the cocoa butter TAG profile and corresponding contents as: POP=24.55, POS=43.63 and SOS=31.80.

At higher pressures, the solubility of water in the SC-CO₂ is higher, shifting the distribution of water from the enzyme to the SC-CO₂ and drying out the enzyme. On the other hand with increased pressure, the solubility of the substrates in the SC-CO₂ phase increases substantially but decreases in the enzyme phase in which the catalysis takes place, causing dilution of the substrate. These factors result in the retardation of esterification (Nagesha et al. 2004).

Higher temperatures result in higher solubility of water in the SC-CO₂ and, thereby lower water activity of the enzyme. Higher temperatures also contribute to the partitioning of the substrates in SC-CO₂ phase and enzyme phase. Optimal diffusion is beneficial for mass transfer between substrates and products (Shishikura et al. 1994).

Kim et al. (2004) reported that longer reaction times resulted in increased acyl migration, by which, the content of major TAGs of cocoa butter analog decreased. As oleic acid constitutes *ca.* 90 % of fatty acids at sn-2 position of TAGs of cocoa butter, increased acyl migration increases

other fatty acids at sn-2 position of TAGs of cocoa butter analog and causes the reduced yield of POP, POS and SOS.

Enzymes require a limited amount of water to function as a catalyst. Excessive amount of water around the enzyme may inactivate enzyme by creating a barrier between them and the reaction medium (Liu et al. 2007). On the other hand water content is an important parameter that modulates the equilibrium between hydrolytic and synthetic processes. At higher water content, the degree of hydrolytic process increases and causes lower production of TAGs as the synthesis process product (Martinez et al. 2002).

Conclusion

Artificial neural network and genetic algorithm were used to determine the optimal conditions of cocoa butter analog synthesis. Response surfaces methodology was used to design the experiments and data for the neural network training was collected. The general regression neural network model was used to predict POP, POS and SOS content of cocoa butter analog in the genetic algorithm population. The optimal conditions to achieve the most similar cocoa butter analogue to the natural cocoa butter were determined to be 10 Mpa pressure, 40 °C temperature, 4.5 h time, 13 % water and 0.6:1 SSS/CHF ratio. Artificial neural networks can be applied as an effective method to model food processing systems and the trained neural network can be used to evaluate the fitness function of a genetic algorithm optimization.

References

- Ciftci ON, Loglu SF, Ggüs F, Güven A (2008) Prediction of a model enzymatic acidolysis system using neural networks. *Grasas Aceites* 59(4):375–382
- Cravener TL, Roush WB (2001) Prediction of amino acid profiles in feed ingredients: genetic algorithm calibration of artificial neural networks. *Anim Feed Sci Tech* 90:131–141
- Hartmann T, Meyer HH, Scheper T (2001) The enantioselective hydrolysis of 3-hydroxy-5-phenyl-4-pentenoic acid ethylester in supercritical carbon dioxide using lipases. *Enzyme Microb Tech* 28(7–8):653–660
- Jackson MA, King JW (1997) Lipase-catalyzed glycerolysis of soybean oil in supercritical carbon dioxide. *J Am Oil Chem Soc* 74 (2):103–106
- Kadim IT, Mahgoub O, Al-Maqbaly RS, Annamalai K, Al-Ajmi DS (2002) Effects of age on fatty acid composition of the hump and abdomen depot fats of the Arabian camel (*Camelus dromedarius*). *Meat Sci* 62(2):245–251
- Kim IH, Ko SN, Lee SM, Chung SH, Kim H, Lee KT, Ha TY (2004) Production of structured lipids by lipase-catalyzed acidolysis in supercritical carbon dioxide: effect on acyl migration. *J Am Oil Chem Soc* 81(6):537–541
- Koc AB, Heinemann PH, Ziegler GR (2007) Optimization of whole milk powder processing variables with neural networks and

- genetic algorithms. *Trans IChemE, Part C, Food Bioprod Process* 85(C4):336–343
- Kumar TVS, Sankar KU, Divakar S (2011) Synthesis of thymol glycosides under SCCO₂ conditions using amyloglucosidase from *Rhizopus* mold. *J Food Sci Technol*. doi:10.1007/s13197-011-0385-1
- Liu KJ, Cheng H, Chang R, Shaw J (1997) Synthesis of cocoa butter equivalent by lipase-catalyzed interesterification in supercritical carbon dioxide. *J Am Oil Chem Soc* 74(11):1477–1482
- Liu KJ, Chang HM, Liu KM (2007) Enzymatic synthesis of cocoa butter analog through interesterification of lard and tristearin in supercritical carbon dioxide by lipase. *Food Chem* 100:1303–1311
- Martinez JL, Rezaei K, Temelli F (2002) Effect of water on canola oil hydrolysis in an online extraction-reaction system using supercritical CO₂. *Ind Eng Chem Res* 41(25):6475–6481
- Moghaddam MG (2010) Artificial neural network modeling studies to predict the yield of enzymatic synthesis of betulinic acid ester. *Electron J Biotechnol* 13(3):1–12
- Mojovic L, Marinkovi SS, Kuki G, Novakovi GV (1993) *Rhizopus arrhizus* lipase-catalyzed interesterification of the midfraction of palm oil to a cocoa butter equivalent fat. *Enzyme Microb Tech* 15:438–443
- Nagesha GK, Manohar B, Udaya Sankar K (2004) Enzymatic esterification of free fatty acids of hydrolyzed soy deodorizer distillate in supercritical carbon dioxide. *J Supercrit Fluid* 32(1–3):137–145
- Rafieian F, Keramat J, Kadivar M (2011) Optimization of gelatin extraction from chicken deboner residue using RSM method. *J Food Sci Technol*. doi:10.1007/s13197-011-0355-7
- Rivera EC, Costa AC, Maciel MRW, Filho RM (2006) Ethyl alcohol production optimization by coupling genetic algorithm and multilayer perceptron neural network. *Appl Biochem Biotechnol* 129–132:969–984
- Ruhil AP, Singh RRB, Jain DK, Patel AA, Patil GR (2011) A comparative study of kinetic and connectionist modeling for shelf-life prediction of Basundi mix. *J Food Sci Technol* 48(2):204–210
- Shishikura A, Fujimoto K, Suzuki T, Arai K (1994) Improved lipase-catalyzed incorporation of long-chain fatty acids into medium-chain triglycerides assisted by supercritical carbon dioxide extraction. *J Am Oil Chem Soc* 71(9):961–967
- Silroy S, Sengupta A, Bhattacharyya DK, Ghosh M (2011) Optimization of reaction parameters of acidolysis reaction between mustard oil and capric acid by using *Thermomyces lanuginosus* lipase. *J Food Sci Technol*. doi:10.1007/s13197-011-0543-5
- Specht DF (1991) A general regression neural network. *IEEE Trans Neural Netw* 2(6):568–576
- Sridhar R, Lakshminarayana G, Kaimal TNB (1991) Modification of selected Indian vegetable fats into cocoa butter substitutes by lipase-catalyzed ester interchange. *J Am Oil Chem Soc* 68(10):726–730