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Optimization and Thermodynamics Studies on Enzymatic Milk Fat Splitting Process using Soybean Lecithin

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Abstract: Lecithin a natural product with unique surface active properties makes it ideal in food processing particularly for fat splitting applications. In the present work the effect of initial fat content, process time, initial enzyme concentration and temperature on splitting of milk was studied using soybean lecithin. The optimum conditions for the maximum percentage fat splitting and unsaturated fatty acid formation were found to be an initial fat content 0.3 g, processing time 90 min, lecithin concentration 5 mL of 3% (v/v) and temperature 40ºC. The maximum percentage fat splitting and unsaturated fatty acid formation were found to be 6.26% (w/w) and 23.24% (w/w) respectively. Activation energy (E_a) required for the milk fat splitting using soybean lecithin was found to be 0.44 J/mol.

Key words: Activation energy, fat splitting, lecithin, optimization

INTRODUCTION

The tissues of an adult human contain relatively constant amounts of protein and carbohydrate. Although there are more than twenty fatty acids occurring in the foods, the common animal body fats are composed chiefly of the glycerides of palmitic, stearic and oleic acids, and in addition generally small amounts of myristic and linolic acids. The fat content may vary within wide limits depending upon the sources. There are series of saturated fatty acids with four to twenty four carbon atoms. The unsaturated fatty acids include those of fourteen to twenty two carbon atoms and of one to six double bonds. Both unsaturated fat and saturated fat are found in a variety of foods, studies have found that these fats are not created equally. Unsaturated fats can be beneficial to your heart, whereas saturated fats could be detrimental to your cholesterol and your heart. Unsaturated fat is liquid at room temperature, differs from other fats in that it contains one or more double-bonds between carbon atoms in its structure. There are two forms of unsaturated fat, monounsaturated fat and polyunsaturated fat (Barnebey *et al*., 1948; David, 1995).

Obesity has been linked to raise incidence of premature death as well as several serious medical conditions, including type-2 diabetes, insulin resistance, heart disease, high blood cholesterol, high blood pressure, and stroke. Obesity is also a risk factor in higher rates of certain types of cancer, as well as fatty liver disease, vascular disorders, thrombosis, obstructive sleep apnea, musculoskeletal problems and gastroesophageal reflux. Abdominal obesity is associated with insulin resistance syndrome and cardiovascular disease (Schwartz *et al*., 1962; Wallnoefer *et al*., 1973; Golay and Bobbioni, 1997; Bruha *et al*., 2000; Romieu and Lajous, 2009).

Lecithins are biologically active additives to food and forages are created concerns to valuable components of soya oil. Lecithin is phosphatidylcholine. It is widely widespread phospholipid of cellular membranes and is a part of a brain fabric of the person and animals. Lecithin present in soybeans, seeds of sunflower, and germs of wheat. Soybean lecithin is a complex mixture of phospholipids, glycolipids, triglycerides, sterols and small quantities of fatty acids, carbohydrates and sphingolipids. Soya lecithin has been popular through the years as a supplement taken for the purpose of fighting cholesterol, supporting liver health, and promoting weight loss (Brook *et al*., 1986).

Phosphatidylcholine facilitates the emulsification of fat into the tiniest particles within the nanosphere, enabling the absorption and transportation of fat. After

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subcutaneous injections of phosphatidylcholine into fat tissue, the adipocytes burst and phosphatidylcholine increases the secretion of triaglycerolrich lipoproteins, which leads to the dissolution of fat by producing an emulsion of nano sized monoglycerides that is transported into the liver and metabolized by beta-oxidation, in the citric acid cycle. Posphatidylcholine is also known to protect the liver through the regeneration of liver cells in cases of fat liver hepatitis and alcoholic hepatic steatosis. In the lungs and inner organs, phosphatidylcholine works as a superficially active substance that prevents alveolar collapse at the end of respiration. The choline and inositol in lecithin protect against hardening of the arteries and heart disease by promoting normal processing of fat and cholesterol. Lecithin itself helps to bind fats and cholesterol to water so that they can pass through the body rather than cause a potentially harmful buildup in the heart or liver. Lecithin breaks up the bad cholesterol in our blood and prevents sediments of fats, and so lowers blood pressure and the chances of a heart attack (Melvin *et al*., 1963; Deuticke *et al*., 1981; Mathur *et al*., 1996; Rittes, 2001; Rotunda *et al*., 2004; Hasengschwandtner, 2005).

Fat splitting is generally defined as the process of obtaining fatty acids from triglycerides by using water at high temperatures and high pressure or by catalyzing the action of the water at relatively low temperature with an acid, alkaline or enzyme as catalyst. Fat splitting may be classified as acid splitting, basic splitting, continuous high temperature splitting, and enzymatic splitting (Muckerheide, 1952). Enzymatic fat splitting is advantageous because it operates at mild conditions and more specific in reaction. The rate of enzymatic fat splitting is depends upon the various factors. Establishing the optimum conditions and understanding the activation energy are important to analyze the characteristics of the enzymatic reactions.

In the present work the effect of process parameters like initial fat content, process time, initial enzyme concentration and temperature were studied and optimized for the maximum splitting of milk fat using soybean lecithin. The activation energy required for milk fat splitting reaction and thermal stability of lecithin were also studied.

MATERIALS AND METHODS

Milk fat: Milk fat (milk butter) was purchased from local market at Chennai in India was used in this study. The composition of the milk fat was analyzed using gas chromatography. It was found that the fresh milk butter consist of saturated fat 67.35% (w/w), unsaturated fat 32.61% (w/w) and moisture content 0.04% (w/w).

Soybean Lecithin: The soybean lecithin used in this study was purchased from Sigma-Aldrich Corporation, Bangalore, India.

Estimation of fatty acid content: About 0.25 gm of fat was transferred into a 250 mL ground-necked round bottom flask with reflux condenser. 10 mL of 0.5N sodium hydroxide in methanol and the boiling chip were added. The solution was boiled for 15 min. The condenser was removed after reflux stops. 2 drops of phenolphthalein was added to the flask. 1 N sulphuric acid was added until the solution becomes colorless and 1 ml in excess. The content was extracted with pet ether and evaporated to dryness. 20 mL of sulphuric acid in methanol was added and the content was boiled for 20 min with condenser. Then the flask was cooled in running water. The content was extracted with pet ether thrice and ether layer was washed with water three to four times. The ether layer was passed through anhydrous sodium sulphate and evaporated to dryness. Reconstituted in pet ether and the fatty acids content was analyzed using gas chromatography (BIS IS 548-3, 1976).

Study on optimization of milk fat splitting using Soybean Lecithin: The effect of various process parameters namely initial milk fat content, initial enzyme concentration, process time and temperature was studied on enzymatic milk fat splitting process. The effect initial fat content on fat splitting process using soybean lecithin was studied by conducting experiments at different initial fat contents varied from 0.1, 0.2, 0.3, 0.4 and 0.5 g, for different process time like 30, 60, 90, 120 and 150 min and using different initial enzyme concentration namely 1, 2, 3, 4 and 5% (v/v) by keeping other parameters constant. The effect of temperature on milk ft splitting and thermal stability of lecithin enzyme was studied for varied temperature at 25, 30, 35, 40, 45 and 50ºC while keeping other parameters constant at initial enzyme concentration of 0.3%, process time of 90 min and initial milk fat of 0.3 g.

RESULTS AND DISCUSSION

Effect of initial fat content on milk fat splitting: The effect initial fat content on fat splitting process using soybean lecithin was studied by conducting experiments at different initial fat contents varied from 0.1 g to 0.5 g using 2% (v/v) initial lecithin concentration for 90 min process time at a temperature of 40ºC. The composition of the fat samples before and after lecithin enzyme treatment was analyzed gas chromatography. The results obtained on the effect of initial fat content on milk fat splitting process using soybean lecithin is shown in Fig. 1. As the initial fat content was increased from 0.1 to 0.3 g the percentage fat split was found to increase and the percentage unsaturated fatty acid (USFA) formed also increased. Further increase in initial fat content did not produce more USFA and the percentage of fat split was

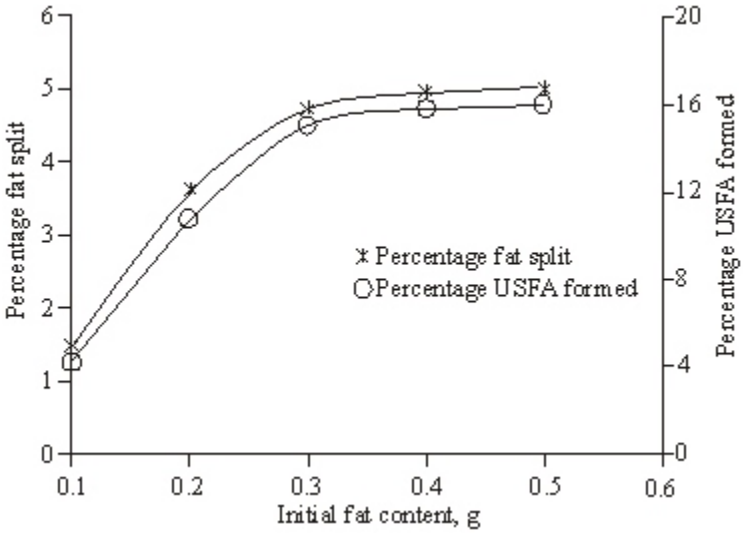

Fig. 1: Effect of initial fat content on milk fat splitting using soybean lecithin at constant process time 90 min, lecithin concentration 2% and temperature 40°C

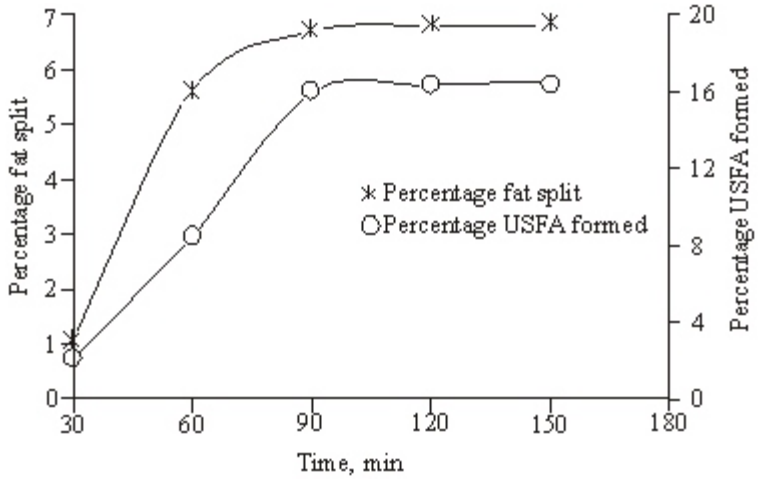

Fig. 2: Effect of contact time on milk fat splitting using soybean lecithin at constant initial fat content 0.3 g, lecithin concentration 2% and temperature 40°C

not increased. This may be due to the lesser initial lecithin enzyme concentration. The maximum percentage milk fat split of 4.72% and percentage USFA of 15.03% were obtained for 0.3 g of initial fat content. Hence the 0.3 g initial fat content was found to be optimum and considered for further studies.

Effect of process time on milk fat splitting: The effect fat split process time on milk fat splitting using lecithin enzyme was studied by conduction experiments for different process time 30, 60, 90, 120 and 150 min, respectively while keeping other parameters constant at temperature 40°C and initial milk fat content 0.3 g with an enzyme concentration 2%. The results on the effect of process time on milk fat splitting are given in Fig. 2. As the process time increases the percentage fat split and USFA formed were found to increase. The maximum percentage fat split and USFA formed was observed at 90 min of process time. The percentage fat split and USFA formed did not increase for further increase in fat splitting process time. This may be due to the non-availability of active lecithin enzyme in the later stages of fat splitting. The optimum fat splitting process time was found to be 90 min. Hence an optimum process time of 90 min was used for further studies.

Effect of initial lecithin enzyme concentration on milk fat splitting: The effect of initial enzyme concentration

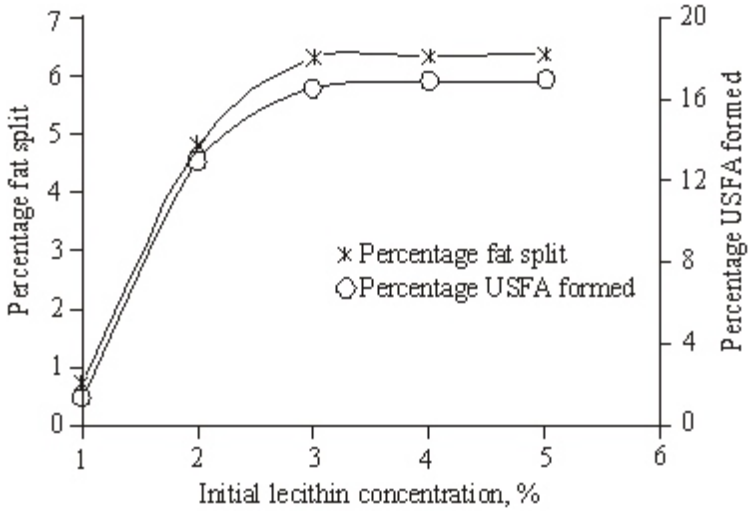

Fig. 3: Effect of soybean lecithin concentration on milk fat splitting of milk fat at constant initial fat content 0.3 g, process time 90 min and temperature 40°C

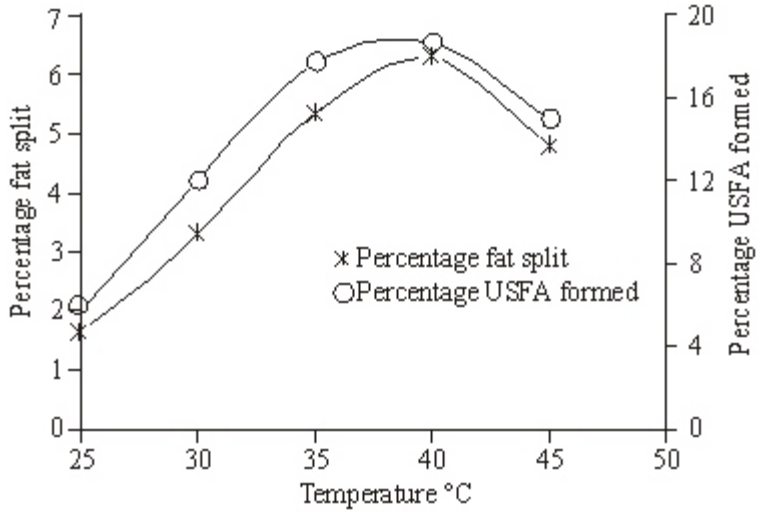

Fig. 4: Effect of temperature on milk fat splitting using soybean lecithin at constant initial fat content 0.3 g, process time 90 min and lecithin concentration 3%

on splitting of milk fat using lecithin enzyme was studied by conducting experiments at different initial enzyme concentration of 1, 2, 3, 4 and 5 with an initial milk fat content of 0.3 g for a process time of 90 min at 40ºC. The result of the effect of initial lecithin enzyme concentration on milk fat splitting process is shown in Fig. 3. As the initial lecithin enzyme concentration was increased the percentage fat splitting and USFA formation was found to increase up to 3% initial lecithin concentration. The percentage fat splitting and USFA concentration did not increase when initial lecithin concentration was increased from 3 to 5%. The maximum percentage fat splitting of 6.26% and the USFA formation of 16.44% was obtained with an initial lecithin concentration of 3% (v/v) and was chosen as the optimum lecithin enzyme concentration for further studies.

Effect of temperature on milk fat splitting: Enzymes are highly sensitive to temperature as temperature increases the enzyme activity is also expected to increase. This concept is followed here for increasing the rate of milk fat splitting. The effect of temperature on rate of milk fat splitting was studied by conducting experiments at different temperatures namely 25, 30, 35, 40 and 45ºC by keeping other parameters constant at an initial fat content of 0.3 g for a processing time of 90 min using 3% lecithin enzyme. The result of the effect of temperature on milk fat splitting is shown in Fig. 4. As temperature increases the percentage fat splitting and USFA formation were found to increase steadily at a faster rate upto 40ºC.For further increase in temperature beyond 40 to 45ºC, the percentage fat splitting and USFA concentration

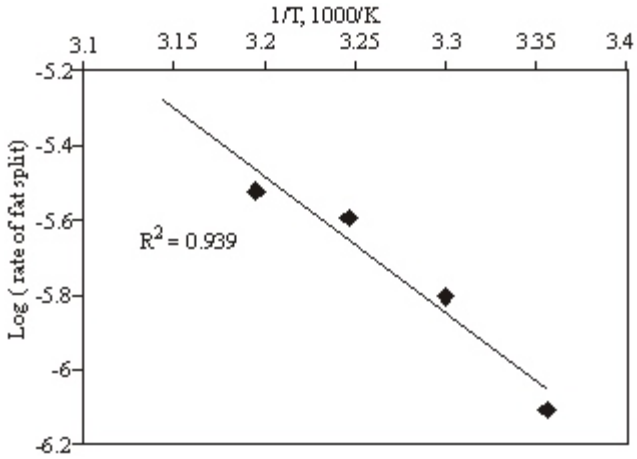

Fig. 5: Arrhenius plot for milk fat splitting using soybean lecithin

was not increased. Hence the optimum temperature was found to be 40ºC which gave a maximum percentage fat splitting of 6.26% and USFA formation of 23.24%. The higher percentage fat splitting at 40ºC is due to the increased activity of lecithin enzyme at higher temperature. At 45ºC the percentage fat splitting was found to be less, this may be due to the deceased activity of enzyme caused due to the thermal denaturation of the enzyme. Figure 5 shows the Arrhenius plot for milk fat splitting process using 3% soybean lecithin at constant initial fat content 0.3 g and for a process time of 90 min. The activation energy required for milk fat splitting process using 3% soybean lecithin was found to be (E_a) 0.44 J/mol.

CONCLUSION

The soybean lecithin was found an effective catalyst for splitting of milk fat. The optimum conditions for the maximum percentage fat splitting were found to be 0.3 g of initial milk fat content, 3% (v/v) initial lecithin enzyme concentration, processing time of 90 min at a temperature 40ºC. The process of milk fat splitting using soybean lecithin was fund to be endothermic and it requires 0.44 J/mol of activation energy for splitting milk fat.

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