

INVESTIGATION ON THE PREPARATION OF ETHOXYLATED
SUCROSE MONOPALMITATE AND EVALUATION OF ITS
POTENTIAL USE FOR MAKING HIGH-PROTEIN BREAD

by

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INTRODUCTION

About two-thirds of the world's population suffer from malnutrition, especially protein deficiency (1). It has been emphasized that the addition of high biological value protein to the diet would greatly relieve that problem.

Over half of the world's population depends on cereals, especially wheat. Bread is an almost universally accepted food item and is considered ideal for protein supplementation because of its high consumption, acceptability, and low price. Since breadstuffs are staple foods in many countries, it is desirable to fortify wheat flour with protein-rich stuffs to raise protein content and to balance essential amino acids of bread. Of all protein-rich stuffs now available, soy flour merits consideration for fortifying wheat flour to produce high-protein bread. Addition of soy flour in the bread formula is advantageous not only because of its low cost, availability, and high protein content, but also because it contributes lysine, the first limiting amino acid in wheat protein to bread (2).

Unfortunately, the addition of soy flour in breadmaking can induce adverse effects on dough properties and bread qualities (3). It is necessary to add certain surfactants in wheat flour, soy flour, and other ingredients during mixing. Using those surfactants permits adding up to 16% soy flour to bread formula without losing physical qualities (4).

Surfactants, such as monoglycerides, ethoxylated monoglycerides, calcium stearoyl-2 lactylate, sodium stearoyl-2 lactylate, etc. are now widely used in the baking industry (4-10).

By examining the effectiveness of two sets of surfactants, the first set being sorbitan monostearate (SPAN) and its polyoxyethylene derivative- polyoxyethylene (20)* sorbitan monostearate (TWEEN), the second set being glycerol monoglyceride and its ethoxylated derivative- ethoxylated monoglyceride, Dubois and Ash found that those ethoxylated derivatives were better surfactants than the originals for making regular bread and soy fortified bread.

By the study of the chemical structures of those two sets of surfactants, both original structures contain ester linkages of monoester and free hydroxy groups. After polymerization with ethylene oxide, those free hydroxy groups will be converted into polyether forms. Physically, the most drastic change of ethoxylated derivatives is solubility in polar solvents, which will be increased.

It is assumed that there are probably more hydrogen bonding sites which are ether linkages available for protein interaction, therefore, they are probably good for making high-protein bread.

The present study was undertaken to ethoxylate sucrose monopalmitate (SMP) and compare the improving action of ethoxylated sucrose monopalmitate (ESMP) with that of sucrose monopalmitate for making high-protein bread.

* Number of oxyethylene units

REVIEW OF LITERATURE

Fatty Acid Esters of Sucrose. Sucrose is the lowest cost hydrophilic grouping available for use in the preparation of nonionic surfactant. Sucrose also is the pure organic compound which is produced in the greatest quantity- about 10,000,000 tons per year (11).

In 1860, Berthelot (12) heated sucrose and stearic acid together and failed to obtain an ester. The history of sucrose esters began in 1880. In an article in the *Berichte der Deutschen Chemischen Gesellschaft*, Herzfeld described sucrose octaacetate, a crystalline compound of intensely bitter taste which eventually found a minor use in denaturing alcohol. Hess (13), in 1921, treated sucrose with stearyl chloride in quinoline to obtain the octastearyl sucrose. Rosenthal, in 1924, prepared the highly substituted sucrose esters of dry oil fatty acids. The route chosen was the classical condensation of the acid chloride in the presence of pyridine. Harris (14) referred to the stearic acid ester of sucrose obtained by heating together equimolar quantities of sucrose and stearyl chloride in pyridine. Lorand (15) used the reaction of sucrose with palmitic anhydride in the presence of monochloroacetic acid and magnesium perchlorate to obtain a product. Rheineck et al., in 1937, took out U. S. 2,077,371, used the same approach but utilized, in addition to sucrose, several alternative polyhydroxy compounds such as mannitol. It was not commercially successful because of poor economics and dark color of the products.

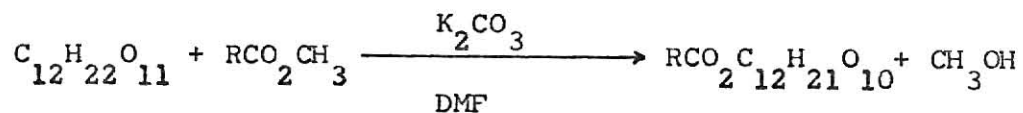
Zief (1950) described the formation of the sucrose octaesters of six unsaturated acids: Methacrylic, Crotonic, Cinnamic, Furoic,

Undecylenic, and Allyloxycarbonic (16). The acid chlorides were used with pyridine. All the octaesters were obtained in satisfactorily pure form. The Methacrylic derivatives polymerized most rapidly of the six with the Crotonic and Allyloxycarbonic esters being of intermediate activity.

Osipow et al. developed a process for the preparation of fatty acid esters of sucrose using the transesterification reaction, which was published in 1956 as the Snell process (17). The process was based on the reaction of sucrose with a glyceride or other ester fatty acids. The idea which made this practical was the use of a relatively new solvent, dimethyl formamide. The theory behind this is that a surfactant should have a water-soluble end and an oil-soluble end called hydrophilic and lipophilic, respectively.

In 1959, Japan's Dai Nippon Sugar Manufacturing Company, Ltd. achieved the first commercial production of sucrose mono fatty esters incorporating the Snell process (18).

In the Snell process (17), the products that were processed from glycerides gave a serious analytical problem because they contained highly complicated and almost insoluble mixture of mono-, di-, and triglycerides and mono-, di-, and higher esters of sucrose. Later on, it was realized that the process could be carried out faster if methyl esters of fatty acid were used and the methanol measured as it distilled off:



Alkaline catalysts were required but heating sucrose with alkali would result in forming salts of lactic acid. The problem

was eventually solved by using rigorously anhydrous conditions, temperature around 90°C, and a partial vacuum which encouraged distillation of methanol.

When making sucrose esters, a trace of water can cause a considerable amount of degradation and brown color. This can be prevented by distilling off the water from the sucrose-formamide solution under vacuum through a rectifying column returning the DMF. Potassium carbonate emerges as the catalyst of choice. When it reacts with sucrose, it forms only half as much water as would be the case with potassium hydroxide and it is a much milder and less expensive base than potassium or sodium methoxide which can also be used:



The solvent, dimethyl formamide, is also not stable to aqueous base:



After reaction, methanol is distilled out of the solution. The DMF is recovered by distillation and the unreacted sucrose is extracted in dilute brine from the ester in the solvent. The product is freed of residual solvents and dried to yield the useful sucrose esters.

There are problems specific to the materials under consideration here. The solubility of sucrose is entirely different from that of fatty acids. Sucrose is sensitive to heat and acid. There are eight hydroxyl groups in sucrose; three primary and five secondary, all of which can react. In a single run, theoretically

255 different sucrose esters, including isomers, can be formed. It could be a Herculean task to separate the esters of even a single fatty acid into the individual pure components (19).

There are several patents for the preparation of sucrose monoesters claimed by Curtis (1961), U. S. patent 2,999,858 (20) and D'Amato (1962), U. S. patent 3,054,789 (21). Recently, in the Southern Regional Laboratory, Osipow et al. (1966) found another way to prepare sucrose ester by the micro emulsion process (19).

Feuge et al. (1969) invented the solvent-free interesterification of molten sucrose and fatty acid esters at temperatures between 170-187°C with the aid of lithium, sodium, and potassium soaps as catalysts and solubilizers (22).

Structure of Sucrose Monoester and Ester Migration. The structural formula of a typical mono-fatty acid ester of sucrose is shown in Fig. 1 (23):

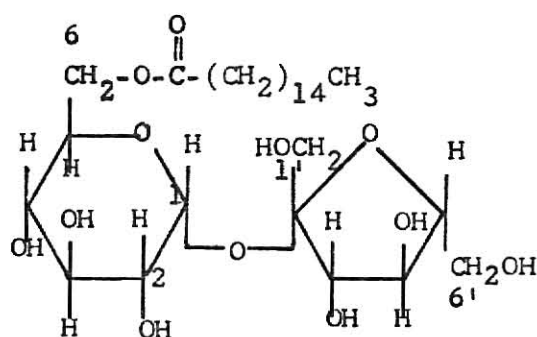
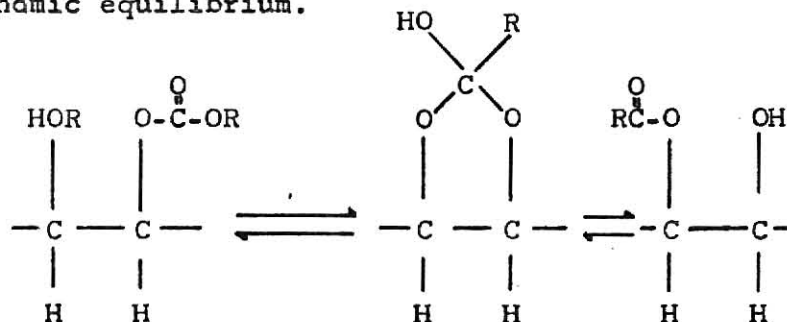


Fig. 1 Chemical structure of sucrose monoester.

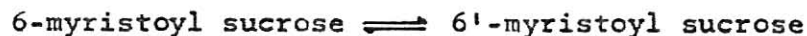
The ester group is attached to the 6 carbon atom of glucose, but about an equal number are located on the 6' carbon of fructose with a smaller number on the 1' and 2 carbon atom (23). York et al. (1956)

pointed out that a product corresponding to sucrose monolaurate was prepared by the alcoholysis of methyl laurate with sucrose. This product appears to be a mixture, with the principal component having the lauroyl radical at the 6-position of the glucose portion (24).

The ability of ester groups to migrate from one hydroxyl to another on an ester of polyol in carbohydrates and inositol field was found by Bonner (25). The rearrangements are believed to occur intramolecularly via cyclic ortho acid intermediate to attain thermodynamic equilibrium.



In Canada, Lemieux and his group first synthesized sucrose by strictly chemical methods and investigated the preparation of sucrose monoesters and the composition of the sucrose monomyristate prepared by transesterification (26, 27). They indicated that the myristoyl groups at the 6- and 6'-positions formed as an equilibrium mixture.



The equilibrium constant (K_e) for the reaction was found to be

$$K_e = \frac{0.62}{0.28} = 2.2$$

" This corresponds to a difference in standard free energy of approximately $0.6 \text{ Kcal mole}^{-1}$ at 80°C , which likely arises mainly from the difference in the non-bonded interactions about the ester groupings.

The pyranose ring of the glucose moiety of sucrose exists in the chair form wherein the atoms on neighboring carbons in the ring are in the staggered relationship. Therefore, a staggering of the substituents on the 6-carbon with the substituents on the 5-carbon of the glucose molecule will cause an eclipsing of two of the substituents on the 6-carbon with the hydrogen and oxygen on the 4-carbon. This relationship is depicted in the conformational formula I (Fig. 2).

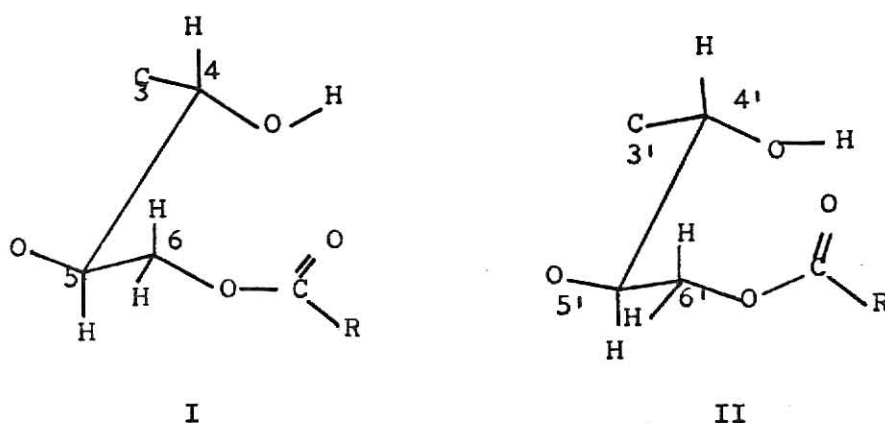


Fig. 2 The conformations of groups or atoms attached to the fourth and fifth carbon atom in the glucose (I) moiety of 6-myristoyl sucrose, and the fructose (II) moiety of 6'-myristoyl sucrose.

On the other hand, the atoms on neighboring carbons in the furanose ring of the fructose moiety can only be in a partially staggered relationship. Therefore, in this case, a staggering of the substituents on carbons 5 and 6 will not eclipse the substituents on the 6-carbon with the hydrogen and oxygen on the 4-carbon (see conformational formula II, Fig. 2). Thus, an acyloxy group at the 6'-position of the sucrose molecule will be in a less congested region of the

sucrose molecule than an acyloxy group at the 6-position" as suggested by Lemieux (27).

Reinefeld et al. (1968) found that partial acylation of sucrose by different methods gave the same products, the 6-monoester (d-glucose unit) first, then the 6'-ester; while the 1'-position was much less reactive than the 6 and 6' positions (28).

Japanese chemists Otake et al. (1969) determined the positions of the ester group in sucrose monopalmitates by o-methylation of the esters and NMR spectra and found that the position 6 of sucrose was most easily esterified and the position 6' was the second. The third easiest site was position 3. An ester group was not detected at the 1' position (29).

Structure and Reactivity of Ethylene Oxide. The production of ethylene oxide by reaction of ethylene chlorohydrin with potassium hydroxide was first reported by Wurtz (30).

Ethylene oxide is also called 1,2-oxidoethand, 1,2-epoxyethane, oxirane, and dimethylene oxide; its chemical formula is $\text{CH}_2\text{---CH}_2$ with an oxygen atom bridged between the two carbons.

It is the lowest member of the cyclic ethers, the 1,2-epoxides. Ethylene oxide is a colorless, sweet smelling liquid, its molecular weight is 44.05. Ethylene oxide must be handled with proper care, since it presents both physiological and explosive hazards (31, 32).

The great reactivity of the three-membered cyclic ether, ethylene oxide, may be attributed to its highly strain nature. Under acidic, basic, and even neutral conditions, the epoxide or oxirane ring can be ruptured readily at the carbon oxygen bond (33-36).

Angle strain-defined as the distortion of bond angles from

their most favored orientation, serves to increase the internal energy of a molecule. Those molecules in which the skeletal bond angles are closest to the preferred value will therefore have the lowest internal energy. Strain energy of ethylene oxide was determined by Cox to be about 28 Kcal/mole (37).

The strain nature of the ethylene oxide ring is immediately apparent from the bond lengths and angles shown in Fig. 3 (38, 39). The bond angle is approximately 30° less than that expected for pure p bonding of the oxygen and approximately 50° less than the C-O-C angle determined experimentally for dimethylether (40).

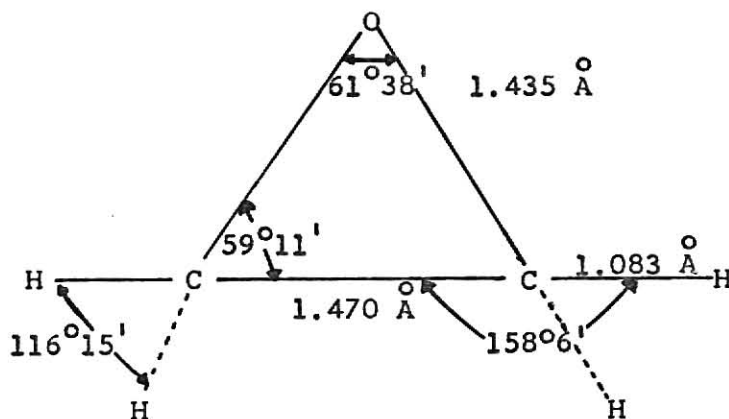


Fig. 3 Bond lengths and angles for ethylene oxide.

The carbon-carbon bond length is intermediate between that of a normal single bond (1.55 Å) and a normal double bond (1.35 Å). Each of the CH_2 group lies in a plane perpendicular to the plane of the ring.

The double bond character of ethylene oxide is obviously like that of cyclopropane. As suggested by Coulson and Moffitt (41, 42), the ring bond of cyclopropane is heavily bent. This structure is shown diagrammatically in Fig. 4.

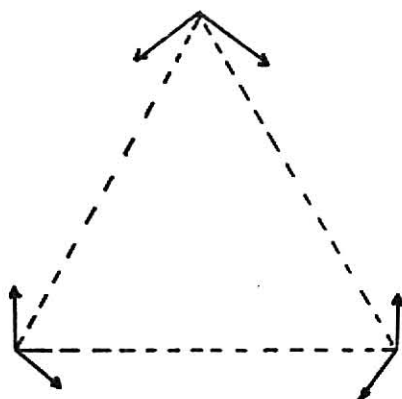


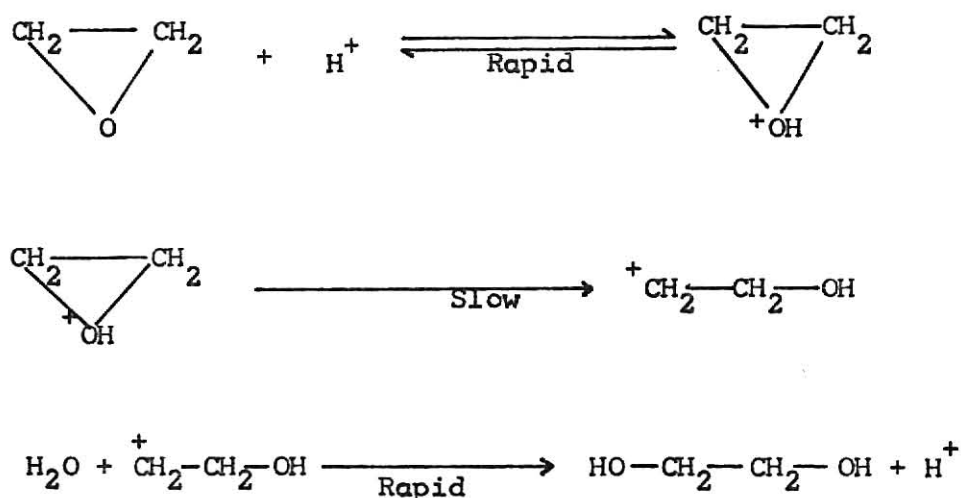
Fig. 4 Bent-bond structure for cyclopropane described by Coulson and Moffitt.

Arrows indicate the direction of hybridized bonding orbitals which may be considered intermediate between sp^2 and sp^3 . The valence atomic orbitals used by the carbon to form the ring bonds contain an unusually large amount of 2P character, and the carbon-hydrogen bonds are rather like ethylene ones. The chemistry of the ether linkage is dominated by the behavior of the $2P_{xO}$ lone-pair electrons. These form the basic site for the addition of protons and other Lewis acids and for the formation of the oxonium salt (43).

Polymerization of Ethylene Oxide. It is possible in many instances to go directly from a heterocyclic monomer to a linear high polymer by a process of ring opening polymerization. The polymers are condensation-type, although no small molecule is eliminated and no hydrogen transfer is involved. However, ring-opening polymerization may be catalyzed by reagents which typically polymerize vinyl monomers. Most of these are cationic and anionic (44).

Ethylene oxide is characterized by great reactivity, as can be expected from its structure. The three-membered ring is under great strain and can readily be opened. Ethylene oxide is therefore easily added to compounds having an active hydrogen atom-ethylene

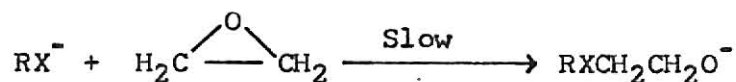
oxide ring opening under acid-catalyzed conditions (35).



However, acid-catalyzed ring opening reaction is rarely used in the production of ethylene oxide polymer adducts.

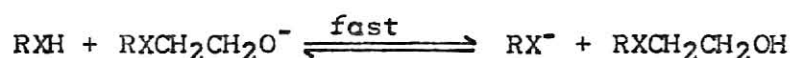
Base-catalyzed ring opening of ethylene oxide is by far the most important type of commercial nonionic surfactant synthesis. The over-all reaction may be divided into two steps; addition of ethylene oxide to the hydrophobic substrate to give the monoadduct, followed by subsequent addition of ethylene oxide in a polymerization reaction (polyoxyethylation) (45). The ring opening reactions of ethylene oxide are classified most generally as nucleophilic substitution (SN) (35, 36).

Under basic conditions, the rate-determining or slow step involved attack of the anion (nucleophile) at a ring carbon atom.

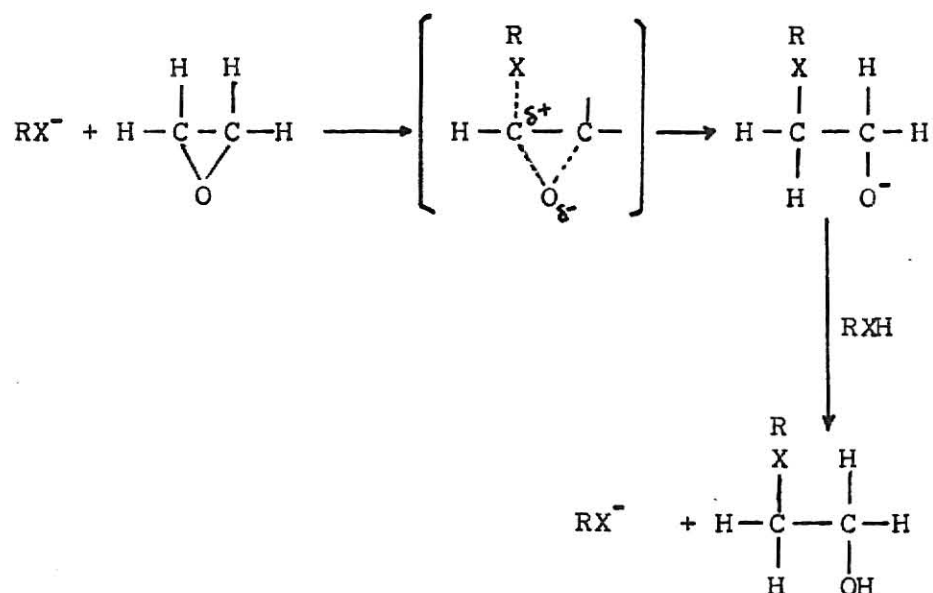


Since the rate is dependent upon $[\text{RX}^-][\text{C}_2\text{H}_4\text{O}]$, it is a second-order nucleophilic substitution, and is designated SN2 (46). In the

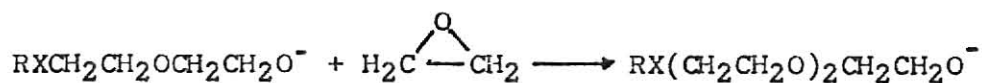
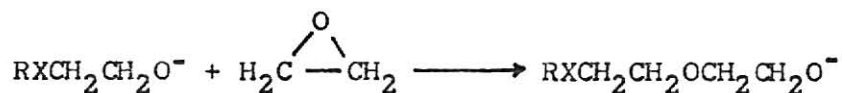
transition state, the C-O bond undergoes scission as the C-X bond is being formed, e. g., bond-making balances bond-breaking and there is no particular charge on the carbon atom.

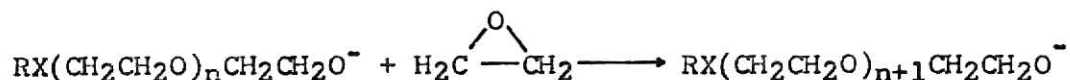


Overall reaction

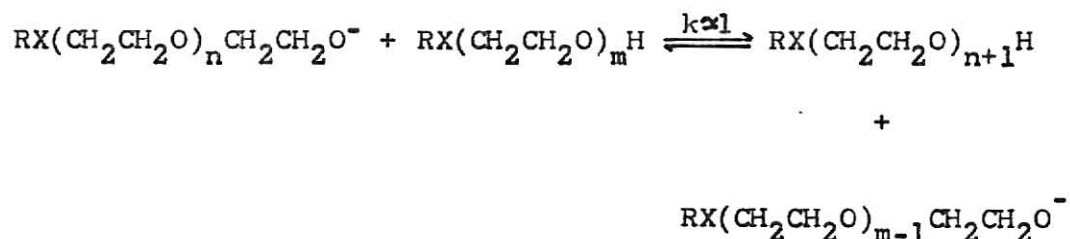


The hydrophilic portion of a nonionic polyoxyethylene surfactant is formed by polymerization of ethylene oxide. The polymerization comprises a series of consecutive $\text{S}_\text{N}2$ ring-opening reactions in which the polyoxyethylene anion acts as the nucleophile:





With minor exception (47-51), each addition of ethylene oxide occurs by a kinetically identical step. This would be expected from the similarity in the structure of the polyoxyethylene anions. Fast proton exchange may then take place with a polyoxyethylene alcohol or with RXH , if any is still present. The acidities of the polyoxyethylene alcohols are nearly identical and independent of chain length (47, 50, 51). Consequently, proton exchange of the type will have no effect on the rate of polymerization, and the relative concentration of anion of a given chain length will depend upon the relative concentration of its conjugate acid in the mixture.



There are no termination reactions. Polymerization continues until all the ethylene oxide has reacted or until the base is destroyed by some other reagent (45).

Small et al. (52) considered the effect of ring size on ΔG° for the heterocyclic compounds. The general shape of the relation between ΔG° and ring size (x) is expected to be similar to that calculated for cycloalkanes (Table 1), particularly if the different atoms does not differ too much from carbon in size and bond angles.

Table 1

Thermodynamical Characteristics of Ethylene Oxide Estimated by Small (52)

Ring size	$-\Delta H^{\circ}$ l.c.	$-\Delta S^{\circ}$ l.c.	$-\Delta G^{\circ}$ l.c.
x	Kcal/mole	cal/deg.mole	Kcal/mole
3	27.0	16.5	22.1

l.c. : liquid condense

Polymerization of ethylene oxide is carried out at elevated temperature under pressure in the presence of catalysts. The reaction is started by heating to a certain temperature, which is then maintained to ensure a satisfactory reaction velocity and product quality. The required quantity of ethylene oxide is added in portions to the reaction mixture. Because of the exothermic nature of the reaction which releases about 22 Kcal/mole ethylene oxide, no further supply of heat is normally required at this stage, and cooling may be necessary. The reaction is allowed to continue at the same temperature until the pressure has dropped sufficiently. The reactor is then cooled and emptied of its contents (53).

Polyoxyethylene polyol ester surfactants are manufactured by the reaction of ethylene oxides with partial fatty esters of polyols. Reaction of glycerol monooleate with ethylene oxide at 100° to 150°C to form a polyoxyethylene monoglyceride was disclosed by Schoeller and

Wittwer (54). Preparation of polyoxyethylene (10) mannitan monostearate, described by Griffin (55, 56), consists of reaction of 10 moles of ethylene oxide with 1 mole of mannitan monostearate in the presence of sodium methylate at 100°C under pressure. After the pressure returns to atmospheric, indicating the completion of the reaction, the product is blown with superheated steam at 10 mm pressure with agitation to free it from residual ethylene oxide. Such products contain oxyethylene chains attached to the site of each hydroxy group of the starting anhydrohexitol ester. The sum of all the oxyethylene chain lengths equals the average number of moles of ethylene oxide attached to each anhydrohexitol unit. Iino (57) studied the preparation of polyoxyethylene sorbitan esters (Tween) at temperatures of 130° to 170°C and indicated that the higher temperature shortened the reaction time. Addition-polymerization of ethylene oxide was performed in an autoclave, whereby reaction temperature was 130-140°C with sodium methoxide as catalyst. Gluzman et al. (58) prepared Tweens from Spans by reacting ethylene oxide with Spans in an autoclave at 125-130°C and a pressure 2-3 atm. with sodium hydroxide as catalyst. The formula of polyoxyethylene (20)* sorbitan monostearate (Fig. 5) illustrates the structure analogy with polyoxyethylene (10) mannitan monostearate.

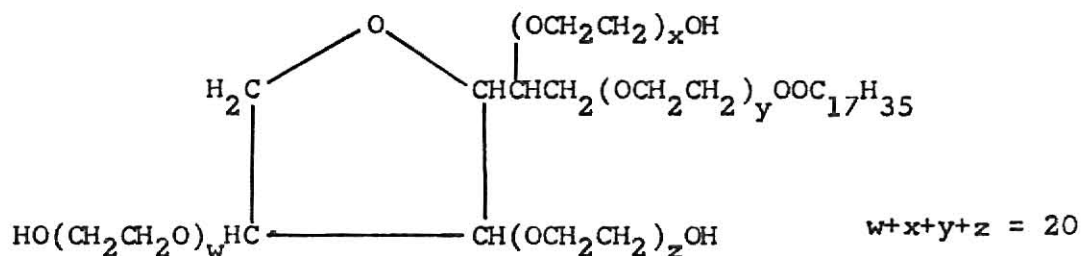
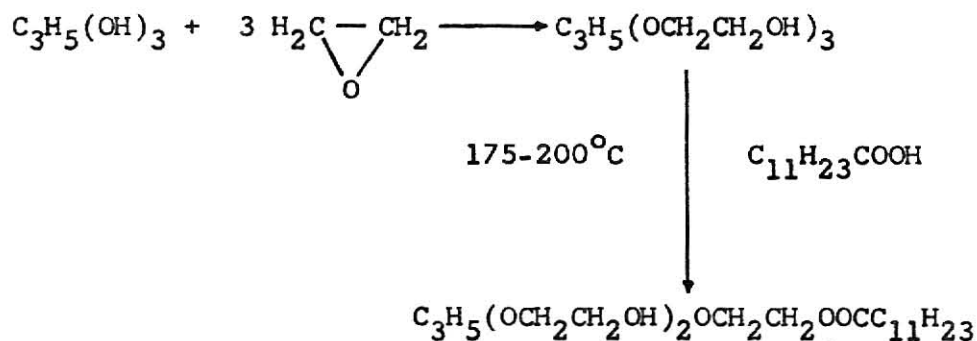


Fig. 5 Chemical structure of polyoxyethylene (20)* sorbitan monostearate.

An alternate method for preparing polyoxyethylene surfactants involves first ethoxylating the polyol followed by esterifying the product with fatty acids, or by using it to alcoholize fatty esters. The monolaurate of the condensation product of 3 moles of ethylene oxide with glycerol was made (59, 60).



Zilkha et al., at Israel, studied various synthetic routes on anionic graft polymerization of ethylene oxide on starch by using dimethylsulfoxide, dimethyl formamide, and tetrahydrofuran as solvents and potassium naphthalene, and p-toluene sulfonic acid as catalyst (61-64).

LeMaistre et al. (65) carried out the reaction of sucrose with ethylene oxide with molar ratios 1, 2, 3, 4, and 11 based on 1 mole of sucrose. It did not appear feasible to isolate the individual components of the reaction mixtures. Reeder et al. prepared a series of pure long chain ethers of sucrose by reaction of sodium sucrate and long chain alkyl bromide in dimethylsulfoxide (66).

In order to eliminate the use of toxic solvents, DMF and DMSO, Duchateau tried to react ethylene oxide with sucrose in suspension in vegetable or animal fat using potassium hydroxide as catalyst without solvent. It was successful in a laboratory scale (67).

Polyoxyethylene Type Nonionic Surfactants Used in High-protein Bread. Surfactants are added to an emulsion to increase its stability by interfacial action. They are divided into two broad categories, ionic or nonionic, according to the character of their colloidal solutions in water (68). Nonionic surfactants play a very important role in the baking industry. Moreover, the surfactant can act as an emulsifier, or react with starch or protein.

For making high-protein bread, the functional problems associated with using soy flours are: effects on water absorption, change in fermentation schedule, effects on the gluten complex, and adverse effects on color and flavor (70). Surfactants incorporate soy flour in bread formula to make high-protein bread. Surfactant molecules have a dual functionality, i. e., they are both hydrophilic and lipophilic. On the polyoxyethylene type nonionic surfactant, the number of ethylene oxide groups added, for a given hydrophobic group, seriously affects the solubility and surface properties of the resulting agent. As a matter of fact, it can be shown that the ability to stabilize certain types of emulsion depends on the relative amount of ethylene oxide added to a given hydrophobic group (71).

Monoglycerides and ethoxylated monoglycerides, sorbitan monostearate and polyoxyethylene sorbitan monostearate (polysorbate 60) have been studied intensively for use in breadmaking or high-protein breadmaking (10, 68-70,72-76). The number of ethylene oxide molecules attached to both monoglycerides and sorbitan monostearate determines the effectiveness of ethoxylated derivatives, and the most effective forms have been found to contain an average of 20 ethylene oxide molecules attached to the mother molecules (10).

Although these surfactants are used to improve the quality of regular or high-protein breads, the mechanism of the surfactants' improving action is still ambiguous.

MATERIALS AND METHODS

Sucrose Monopalmitate. Sucrose monopalmitate was obtained from Dai-Nippon Sugar Manufacturing Company, Limited, Tokyo, Japan. The trade name is Nitto Ester P-1570 and its chemical formula is



Although the name is sucrose monoester, it is actually a mixture of mono-, di-, and tri-palmitates and stearate esters. Following are some characteristics of Nitto Ester P-1570 (77).

I. Properties:

1) Appearance: White or yellowish-white powder through 30 mesh.

2) Fatty acid component:

a) By gas chromatography

<u>Stearate</u>	<u>Palmitate</u>
Approximately 30%	Approximately 70%

b) By thin-layer chromatography

<u>Monoester</u>	<u>Di- and Tri-ester</u>
Approximately 70%	Approximately 30%

3) Softening point: 48-55°C.

4) Thermal stability: It is still unchanged at 130°C, but begins to decompose gradually at 140°C and is completely destroyed at 150°C.

5) Decomposition percentages in 2.5% solution:

<u>100°C</u>			<u>110°C</u>		<u>120°C</u>	
1 hr	3 hr	5 hr	30 min	1 hr	30 min	1 hr
1.9%	3.7%	4.6%	2.4%	3.3%	4.4%	5.6%

6) Decomposition percentages under acidity or alkalinity at
60°C 0.2% solution:

	<u>pH 2-4</u>	<u>pH 8</u>	<u>pH 10</u>
1 hr	98	96	84
2 hr	97	95	78

II. Solubility:

1) In water solution:

Pure water	30°C	SS
	70-100°C	S
1% CH ₃ COOH solution	30°C	D
	60°C	C
75% C ₂ H ₅ OH	30°C	SS
	60°C	S
50% C ₂ H ₅ OH	30°C	SS
	60°C	S

2) In solvent:

Ethanol	30°C	SS
	60°C	S
Methanol	30°C	SS
	60°C	S
n-Butanol	30°C	SI
	60°C	S
Toluene and Benzene	30°C	I
	50°C	S

Ethylether	30°C	I
Ethylacetate	50°C	I
Acetone	50°C	SS
Methylethylketone	50°C	SS
CCl ₄	50°C	S
Chloroform	25°C	S

S= Soluble SS= Slightly soluble

D= Dispersive C= Cohesive

SI= Poor soluble I= Insoluble

III. Nitto ester standard:

Mean molecular weight of fatty acid: 255-285

Total fatty acid combined ester 40-70%

Free sugar not exceeding 10%

Nitrogen not exceeding 0.02%

Acid value not exceeding 5

Ash not exceeding 2%

Drying loss (moisture) not exceeding 4%

Both acetone and methylethylketone can be used for recrystallization of sucrose esters. Methylethylketone is more suitable for monoesters since a smaller volume is required (23). After recrystallization of SMP from methylethylketone, it was placed in a desiccator with P₂O₅ under reduced pressure overnight.

Ethylene Oxide. C₂H₄O, M. W. 44.05, B. P. 10.5-11.5°C. It was obtained from Eastman Kodak Company, Rochester, New York. Hess and Tilton (78) in an account of the handling, explosibility, and toxicity of ethylene oxide classify it as a hazardous chemical. Commercial container is a sealed bottle in a tin can filled with

soft wood. The following procedure for handling ethylene oxide was used:

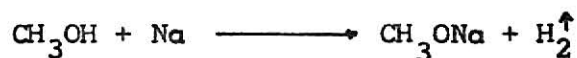
- 1) The tin can was opened carefully.
- 2) The sealed bottle was put into an ice-salt bath for cooling for at least 1/2 hour.
- 3) A gas mask with type A filter or with a special filter must be worn (53).
- 4) Working place must be made safe against explosion. All possibilities of ignition must be eliminated.
- 5) The neck of the bottle was cut very carefully with a file in a place with temperature of around 4°C, such as in a cold box or room.
- 6) Ethylene oxide liquid was transferred to a brown bottle with a septum, kept at a temperature below 0°C until ready for use.

Sodium Methoxide or Sodium Methylate. CH_3ONa , M. W. 54.01.

It serves as a catalyst. Since it is a strong base and absorbs considerable amount of water at room temperature when exposed to air, freshly made sodium methoxide is required. The reaction procedures for sodium methoxide were:

- 1) Methanol (Fisher Scientific Co., Fair Lawn, New Jersey) was distilled over molecular seive # 4A (Grade 51 A 4A^o Fisher Scientific Co.) and the first 50 ml was discarded.
- 2) The redistilled methanol was poured in three flasks and the last one was weighed.
- 3) A piece of metallic sodium was cut and washed in the first two flasks with methanol.
- 4) The previously washed metallic sodium was dumped into the third flask. The reaction started with bubbling (hydrogen gas) and was

exothermic.



- 5) After the bubbling has stopped, the third flask was weighed again to calculate how many grams of CH_3ONa were formed.
- 6) Excess CH_3OH were removed by rotatory evaporator.

N, N-Dimethylformamide (DMF). $\text{H}-\text{CO}-\text{N}(\text{CH}_3)_2$, M. W. 73.09, B. P. $76^\circ/39$ mm, $153.0^\circ/760$ mm. It was obtained from Millinckrodt Chemical Works, St. Louis, Missouri. Since sucrose is insoluble in fats or fatty acid derivatives, a mutual solvent, such as DMF is required to avoid charring (68). In order to get anhydrous condition, a 48 hour treatment procedure described by Ferrari et al. (79) was followed. 10 g of molecular sieves #4A were added for every 100 ml of DMF in the bottle directly and kept tightly stoppered. The molecular sieves were kept in the bottle until the solvent was completely used.

Acetic Acid. CH_3COOH , M. W. 60.05, B. P. 118.5°C . It was obtained from J. T. Baker Chemical Company, Phillipsburg, New Jersey. It served as a neutralizer for CH_3ONa after ethoxylation reaction.



High Pressure Reaction Vessel. The vessel is a number 4522 high pressure reaction apparatus (bomb capacity is 2000 ml) from Parr Instrument Company, Moline, Illinois. The accessories are number 4801 controller, mechanical motor stirrer, and thermocouple.

Nitrogen Gas. It was obtained from Chemetron Corporation, Chicago, Illinois.

Ethoxylation Reaction.

I. Small scale preparation:

- 1) The following chemicals were weighed out:
 - a) SMP 6.123 g (0.01 mole)
 - b) Ethylene oxide 10 ml (0.2 moles)
 - c) Trace amount of sodium methoxide
- 2) Weighed SMP was put in a 500 ml resin kettle, 100 ml DMF added and the mixture stirred constantly. When SMP was completely dissolved, 10 ml liquid ethylene oxide was injected by syringe quickly.
- 3) The cover of the resin kettle (which has a pressure gauge) was tightened with a spring screw. The resin kettle was then put into a paraffin oil bath.
- 4) The temperature of the oil bath was slowly raised to 100°C and this took approximately 1 hour.
- 5) The solvent system in the reaction vessel was stirred with a magnetic stirring bar. The oil bath was stirred with a mechanical stirring rod and its temperature was measured with a thermometer.
- 6) Temperature of the oil bath was kept around $105 \pm 5^{\circ}\text{C}$.
- 7) The pressure gauge was observed. When the reaction started, the pressure gradually increased until a maximum pressure (40-50 psi) was reached, which then went down until it equalized with atmospheric pressure. This meant that the reaction has been completed, with ethylene oxide gas returning to the solvent to react with metal succinate, which was formed by sucrose monopalmitate reacting with sodium methoxide.
- 8) A few drops of acetic acid were added to neutralize

sodium methoxide.

- 9) Precipitation gel formed in the reaction vessel was transferred to a porous filter connected with suction to get light yellow semi-gel solid.
- 10) The filtrate was transferred to a 500 ml round bottomed flask to be distilled by vacuum distillation. DMF was trapped by acetone and dry ice. This took 8-10 hours.
- 11) The solids were combined, dissolved in absolute alcohol, and treated with 5% activated carbon.
- 12) Polyethylene oxide was extracted with a saturated salt-water solution.
- 13) The crude product was recrystallized with ethanol at 0°C to obtain white grey solid form. Traces of ethanol were removed by reduced pressure over P_2O_5 for 24 hours.
- 14) The solid (ESMP I) obtained was ground in a pre-cooled mortar and pestle.

II. Large scale preparation:

- 1) The following chemicals were weighed out:
 - a) Sucrose monopalmitate 61.23 g (0.1 mole, Mean M. W. 612.3)
 - b) Ethylene oxide 88 g (2 moles)
 - c) Sodium methoxide 0.175 g (23)
- 2) Sucrose monopalmitate was dissolved in 500 ml DMF in a 1000 ml beaker and stirred with magnetic stirring bar constantly with temperature around 30-40°C until sucrose monopalmitate was dissolved completely.
- 3) The proper amount of catalyst sodium methoxide was added to the above solution.
- 4) SMP in DMF solution was transferred into a 2000 ml high

pressure bomb which was closed tightly with hand. The bomb was connected to nitrogen gas and flushed with it twice.

- 5) A 300 ml stainless steel cylinder was flushed with nitrogen gas twice and vacuumed for a few minutes until pressure reached around 5-10 cm. The valves were then closed tightly.
- 6) As soon as the vacuum cylinder was ready, ethylene oxide liquid was weighed out in the proper amount in a 250 ml beaker and transferred with a glass funnel into the vacuum cylinder which sucked the liquid ethylene oxide automatically. The surrounding temperature was around 4°C, as in a cold room or cold box.
- 7) One end of liquid ethylene oxide cylinder was connected with nitrogen gas tank through a high pressure metal tubing, and the other end with stainless steel pipe through a singlet glass tube protected by a steel box and heavy wall glass. The end of the pipe was connected with the bomb (reaction vessel) which has a motor mechanical stirrer and thermocouple with controller (entire assembly is shown on Fig. 6).
- 8) The heat control was turned on the controller until the bomb temperature reached 100°C. The nitrogen gas valve was opened. The flow rate control valve was opened gradually and the actual flow rate was observed at the same time through the singlet glass.
- 9) The flow rate of liquid ethylene oxide was adjusted to prevent sudden increase of temperature since the reaction

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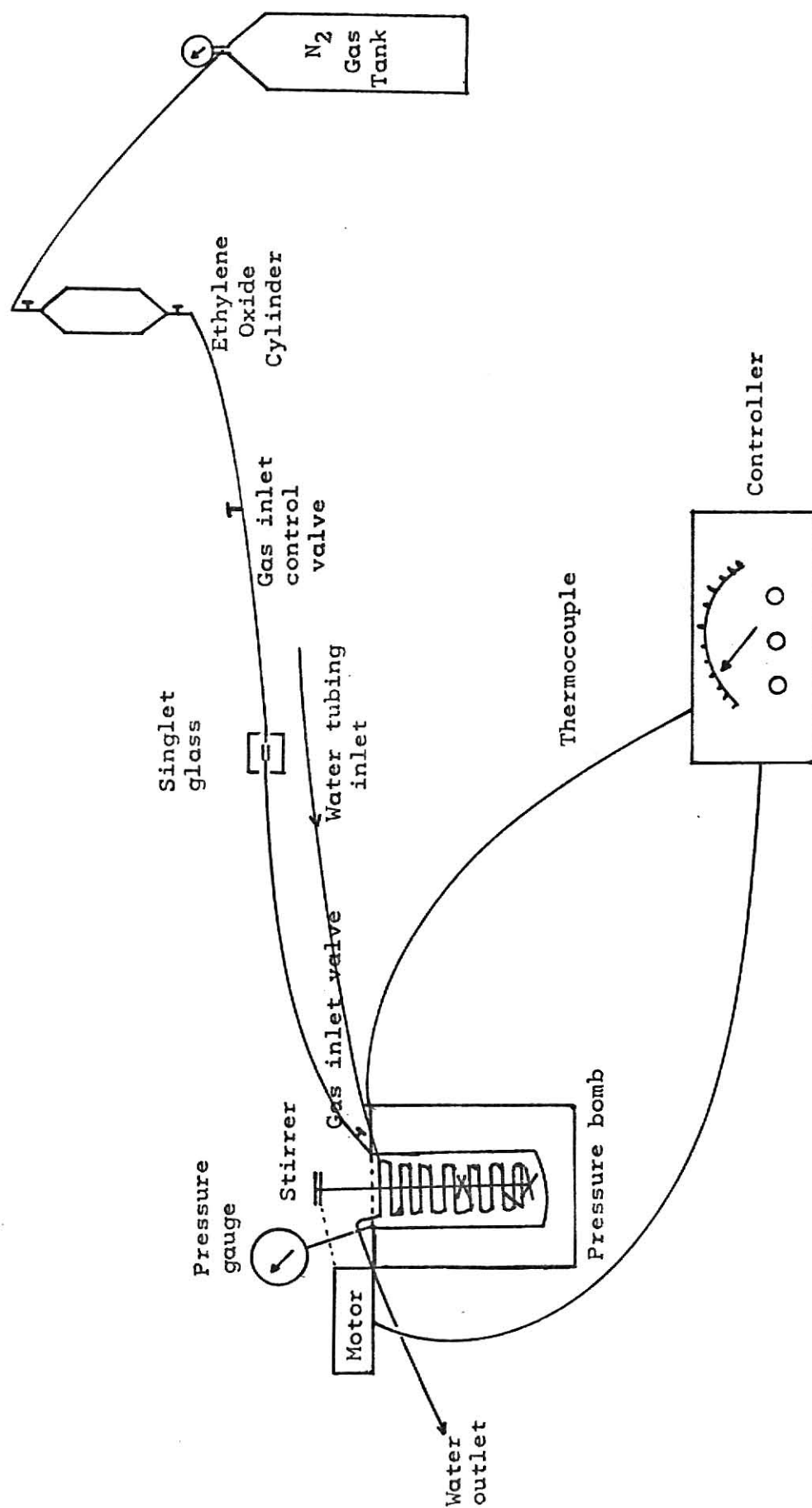


Fig. 6 Assembly of Parr pressure reaction apparatus.

is exothermic. When temperature got too high, it was reduced by cooling the bomb with cooling tubing using tap water.

- 10) Pressure was gradually increased when the reaction started. Temperature was maintained around $105-110^{\circ} \pm 1^{\circ}\text{C}$ throughout the entire reaction.
- 11) The reaction was kept at maximum pressure and constant temperature for 30 minutes. Maximum pressure was maintained by feeding nitrogen gas.
- 12) After 30 minutes, the nitrogen valve was turned off and pressure gradually decreased to 1-2 psi which indicated that the reaction was completed. The total reaction time was $2\frac{1}{2}$ hours.
- 13) When the reaction was completed, the bomb was cooled to room temperature, disconnected and the solution transferred into a 1000 ml round bottomed distillation flask. An equivalent amount of acetic acid was added to neutralize sodium methoxide.
- 14) The vacuum distillation apparatus was set up and the solution was distilled to a syrup (ESMP II). The DMF solvent was collected by acetone and dry ice trapping system.

Isolation and Separation Techniques.

I. Thin-layer chromatography (TLC)

Thin-layer chromatography plates were prepared from silica gel with chloroform as solvent. Plate size was 3 x 1 in. Sucrose monopalmitate and crude product were applied as chloroform solutions, methyl palmitate (Gallard-Schilesinger Chemical Manufac-

turing Corporation) as petroleum ether solution, and polyethylene glycol (M. W. 400, Fisher Scientific, and M. W. 3000-3700, Matheson Coleman and Bell Manufacturing Chemists) as water solutions to apply on TLC by micropeptide. Plates were developed with a mixture of petroleum ether-ether (9:1 V/V) for comparing crude product and methyl palmitate, and also for checking ester bond of crude product after hydrolysis with 3 N HCl. During and after the reaction, sucrose mono-palmitate and crude product were developed by acetone-chloroform (4:1 V/V). After crude product was extracted with saturated sodium chloride aqueous solution (80), more purified crude product and polyethylene glycols were developed in ethanol-chloroform (3:1 V/V). TLC spots were visualized by spraying concentrated sulfuric acid, drying the plates in the air, and then heating them in a hot plate at 110-130°C for a few minutes.

II. Extraction of polyethylene glycol from crude product (80)

A 250 ml separatory funnel was needed for the extraction. Then about 25 g of the sample and 70 ml of sodium chloride solution saturated at room temperature were added to the separatory funnel, which was then immersed to the neck in a boiling water bath. Sufficient time was allowed for the contents to reach about 95-100°C, and then the funnel was removed and vigorously shaken. It was returned to the bath and allowed to stand until the separation of phases was visibly complete. It required 15-30 minutes. The lower brine layer was then separated and discarded. Using the same amount of fresh brine, the extraction was repeated two more times in the same manner.

III. Chemical detection of polyoxyethylene groups in product

Draggendorff reagent (bismuth subnitrate and potassium iodide in acetic acid) gave a positive color test (pink-orange) for product which contains polyoxyethylene groups (81).

Flour. Two commercial wheat flours were used. Wheat flour (Commander) was obtained from Archer-Daniel-Midland Milling Company, Kansas City, Missouri, and was used with small scale preparation product (ESMP I) in baking. It has a protein content of 11.8% ($N \times 5.7$), 0.46% ash, and 11.4% moisture. Wheat flour (Wichita) was obtained from Ross Industries, Wichita, Kansas. Wichita flour was used with large scale preparation product (ESMP II) in baking. It has a protein content of 11.6% ($N \times 5.7$), 0.42% ash, and 13.2% moisture.

Soy Flour. Soy flour (Ardex 550) was defatted and obtained from Archer-Daniel-Midland Milling Company, Kansas City, Missouri. It has 52.6% protein, 6.80% ash, and 6.8% moisture.

Sodium Stearoyl-2 Lactylate (Emplex or SSL). It was obtained from C. J. Patterson Company, Kansas City, Missouri.

Farinograph. Farinograms were obtained by the constant dough weight method as described by AACC method 54-21 A (82), using 50 g farinograph mixing bowl. Water absorptions were adjusted to obtained a dough with a maximum consistency of 500 B. U. When wheat flour was mixed with soy flour (12%) and/or SMP and ESMP (0.25%, 0.5%, 1.0%), the mixture was blended for 2-3 minutes in the farinograph mixing bowl before water was added.

Extensigraph. AACC method 54-10 (82) was used, except that salt was not included in the dough. ESMP (0.25%, 0.5%), SMP (0.25%,

0.5%) and controls were compared in wheat flour and/or wheat flour containing soy flour (12%).

Baking Tests. High-protein bread was baked according to the no-time dough process using optimum baking absorption, potassium bromate and mixing time. The high-protein breadmaking formula was comprised of 600 g wheat flour, 30 g sugar, 12 g salt, 18 g yeast (compressed), bromate level 70 p. p. m., 72 g soy flour, 1.5 g surfactant (ESMP, SMP, SSL), ESMP I being a small scale preparation product. In another baking test, 1.5 g and 3.0 g of surfactant (ESMP, SMP, SSL) was used, ESMP II being a large scale preparation product.

Ingredients were mixed in a Hobart A 200 mixer equipped with a MacDuffee type bowl and fork. After an initial blending for 30 sec. at first speed, the mixing was continued at second speed until optimum dough development. The mixing time was 5 minutes. Water temperature was adjusted to give an optimum dough temperature of 86°F. After mixing, 500 g of dough was scaled, rounded up and given a floor time of 40 minutes at 86°F and 85% humidity in a fermentation cabinet (National Manufacturing Company, Lincoln, Nebraska). Dough was then moulded in a Moline moulder (Model 100, Moline, Inc., Duluth, Minnesota), panned and proofed to template height (1.5 cm) over pan at 96°F and 94% relative humidity. Proofed doughs were baked at 425°F for 25 minutes in an all-purpose rotary oven (Despatch Co., Minneapolis, Minnesota). Loaf weight and volume were measured immediately after baking, usually with 5 minutes.

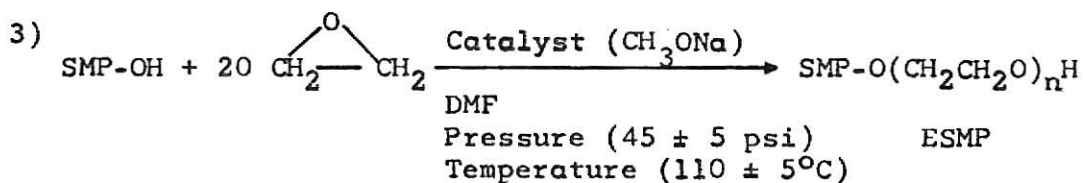
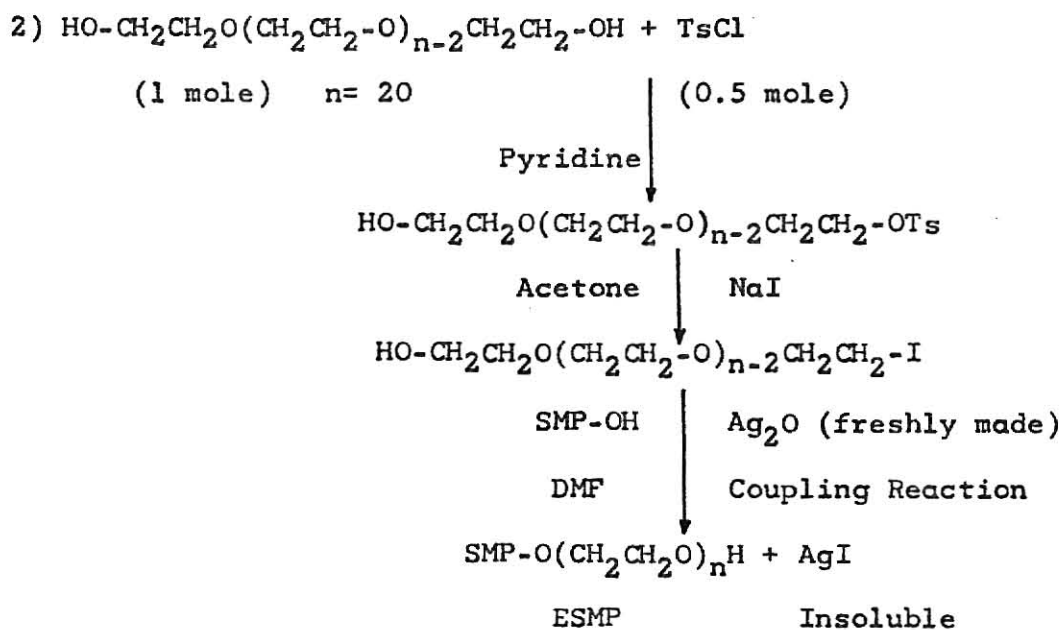
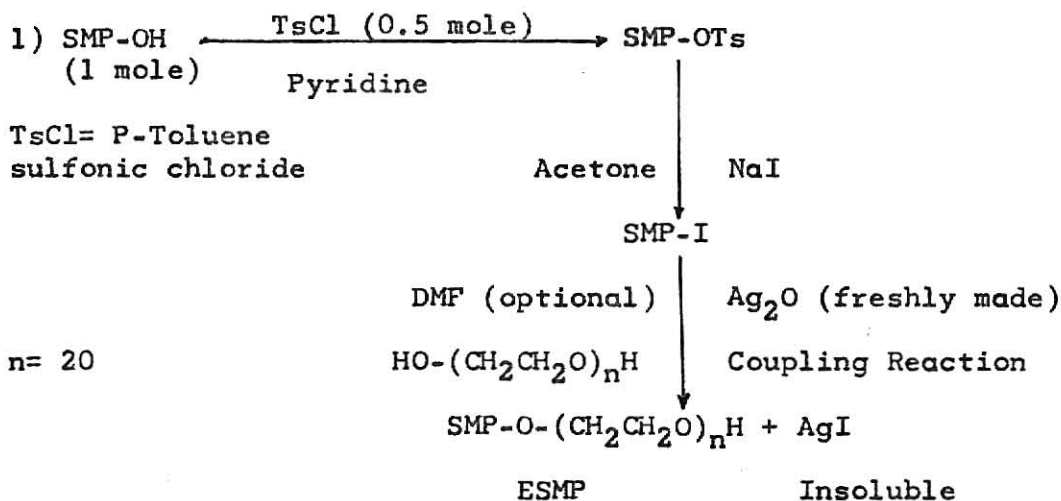
RESULTS AND DISCUSSIONS

Preparation. The distinguished characteristic of ethylene oxide is that the ring can be opened in a single bimolecular reaction, which involves no pre-equilibrium step, to produce good yields of product (83). The individual ether group is less hydrophilic than the individual hydroxyl group. However, even though the hydroxyl group is more hydrophilic, the molecules of polyhydroxy compounds lock numerous hydrogen bridges to form large hard-to-dissolve associates, the intramolecular built-in water acts sometimes as binding sites. On the other hand, the polyether molecules do not possess active hydrogen atoms, but a large number of groups to which strong hydrogen bridges can be formed if molecules with active hydrogen atoms approach from the outside (84). The attempt was thus made to ethoxylate SMP for use as an improver for making high-protein bread.

The overall ethoxylation reaction process could be summarized as follows: Ethoxylation reaction was carried out at elevated temperature under pressure in the presence of catalyst. The reaction was started by heating to $110 \pm 5^{\circ}\text{C}$, which was then maintained to ensure a satisfactory reaction velocity and product quality. The required quantity of ethylene oxide was added in portions to the reaction mixture. Because of the exothermic nature of the reaction which releases about 22 Kcal/mole ethylene oxide, no further supply of heat was normally required at this stage, and cooling might be necessary. The reaction was allowed to continue at the same temperature until the pressure had dropped sufficiently. The reactor was then cooled and emptied of its contents. The ethoxylation of SMP is illustrated

in the block flow diagram in Fig. 7.

Reaction Route. Three possible routes were investigated in the attempt to synthesize ESMP. These were:



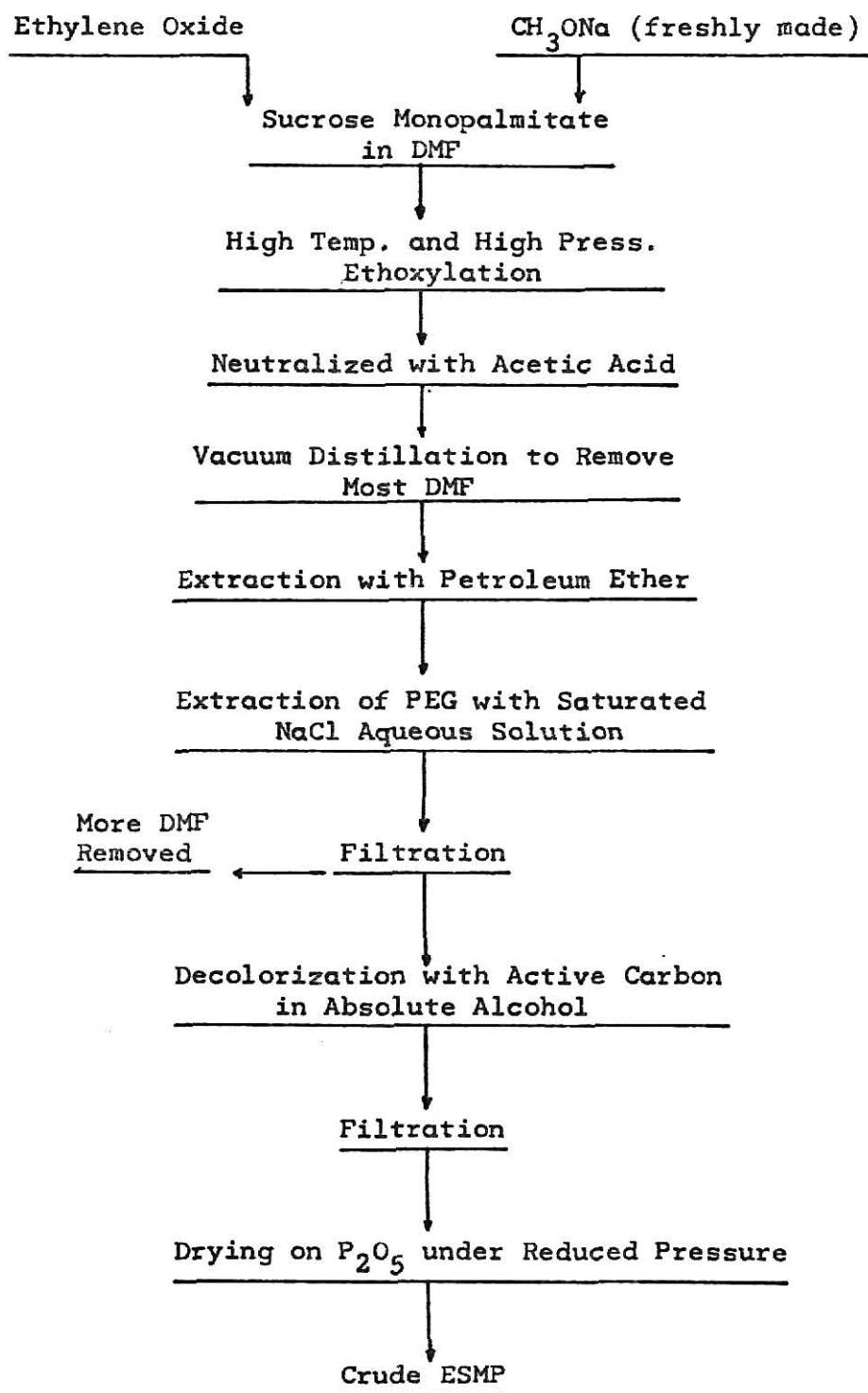


Fig. 7 Flow diagram for the preparation of crude ethoxylated sucrose monopalmitate.

The first route, tosylation of SMP, was achieved (85). Tosyl group of tosylate SMP could be displaced by iodine atom from sodium iodide in acetone solution. Iodo-SMP would then couple with polyethylene oxide with known average oxyethylene unit of about 20. The coupling reaction was aided with the use of silver oxide in the formation of insoluble silver iodide. Tosylation of SMP raised questions of whether there was intramolecular displacement or intermolecular displacement with other sucrose esters. SMP was not highly soluble in acetone. In addition, iodine derivatives were expensive and uneconomical.

The second route was the reverse of the first. Tosylation of known polyoxyethylene (20)* compound was complicated since at least 3 mixtures could be obtained and analytical separation was difficult. As was the case with the first route, the same problem exists with the iodine derivatives being expensive and uneconomical. Both routes involved several synthetic steps to obtain ESMP, but one important advantage is that the average number of ethylene oxide units in the ESMP could be precontrolled.

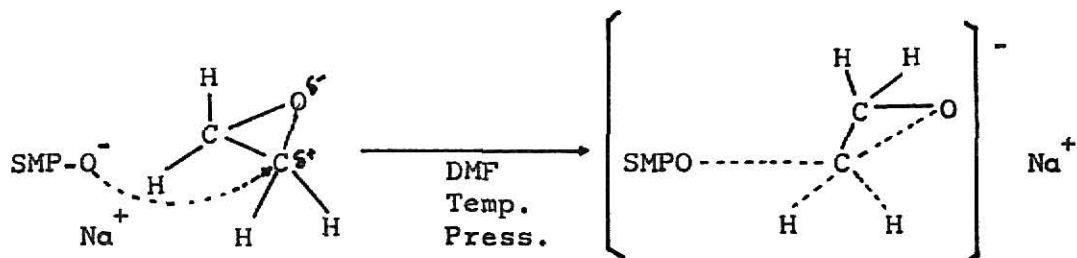
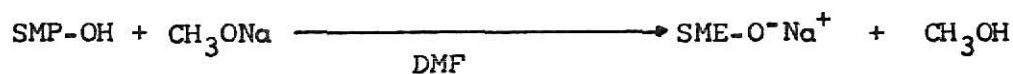
The third route was adapted in the preparation of ESMP, since it was a one step reaction, and the cost of the starting materials was less expensive. The method used was similar to the ethoxylation of sorbitan esters by Iino (57) and Gluzman (58). It is widely used in industrial ethoxylation reaction. However, isolation and separation of reaction mixture were difficult to achieve and it was necessary to get pure ESMP to determine the average number of oxyethylene units in the molecule.

Reaction Mechanism. The reaction mechanism could be proposed as base-catalyzed SN2 nucleophilic polymerization reaction (Fig. 8).

Proposed Reaction Mechanism

An SN2 (Substitution, Nucleophilic, Bimolecular)

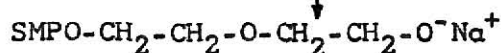
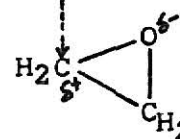
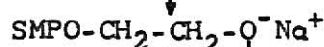
Type of Base-Catalyzed Ethoxylation Reaction



Sodium SMP

Ethylene
oxide

Bond-making balancing
bond-breaking
No particular charge on
carbon atom



Neutralized by CH_3COOH

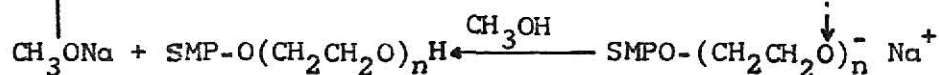


Fig. 8 Diagram of reaction mechanism for SMP ethoxylation.

The cleavage of the carbon-oxygen bond and attack by the nucleophilic occur in a single step. Carbon loses electrons to the leaving group and gains electrons from the nucleophile, and as a result, the carbon atom does not become appreciably positive or negative in the transition state. Because steric factors control reactivity, electronic factors are unimportant (35).

Molar Ratio. The molar ratio of ethylene oxide to SMP was 20:1, since the number of oxyethylene units in ethoxylated monoglyceride and ethoxylated sorbitan monoester (Tween) (10) was found to be most effective in baking when $n = 20$. The fatty acid ester of both ethoxylated derivatives are palmitate and stearate.

Temperature. Reaction rate increases with increasing temperature. The temperature of the ethylene oxide addition reaction varied in the range of 110-220°C and mostly between 130-180°C. Water can be formed at higher temperatures due to the splitting off of the terminal hydroxyl group (53). The temperature was around 100-120°C and reaction time about 2 hours for minimum decomposition of SMP to occur (Table 2).

Table 2

Effect of Temperature on Solution Color of SMP and Ethylene Oxide in DMF in Ethoxylation Reaction.

Temperature	<u>100-120°C</u>	<u>120-140°C</u>	<u>140°C</u>
Color of solution	Light brown	Brown	Dark brown (degradation of sucrose portion)

Pressure. According to mass-action law, an increase in pressure leads to an increase in rate of polyoxyethylation, because ethylene oxide is a gas under the conditions normally employed for the reaction and the rate depends on its concentration. In industrial preparation of polyoxyethylene derivatives, the range is 1.5-5.0 atm. pressure. The pressure, 2.5-3.0 atm. was obtained in both the small and large scale preparation. The relationships between temperature, pressure and time are shown in Fig. 9. In the small scale preparation, the pressure in the resin kettle could be estimated by using the ideal gas law equation $PV = nRT$. The best precaution against the danger of explosion is to dilute gaseous ethylene oxide with nitrogen gas. It was also important that the mixture of gases be free from air, since in the presence of air, an explosion could occur with a much smaller amount of ethylene oxide than would normally be the case.

Catalyst. Although acidic catalysts could be used for ethylene oxide addition reaction, acidic catalysts can cleave glucosidic bond of sucrose monoester molecule to glucose and fructose unit and either unit be attached to a fatty ester. In addition, acidic catalysts gave rise to a larger polyethylene glycol content than basic catalysts. Basic catalysts at about 0.1-0.5% concentration (based on starting material) are normally used, and caustic soda powder, sodium methylate, metallic sodium and caustic potash are some examples (86). The use of a higher concentration does not result in a proportional increase of the addition velocity. The effectiveness of basic catalysts for the

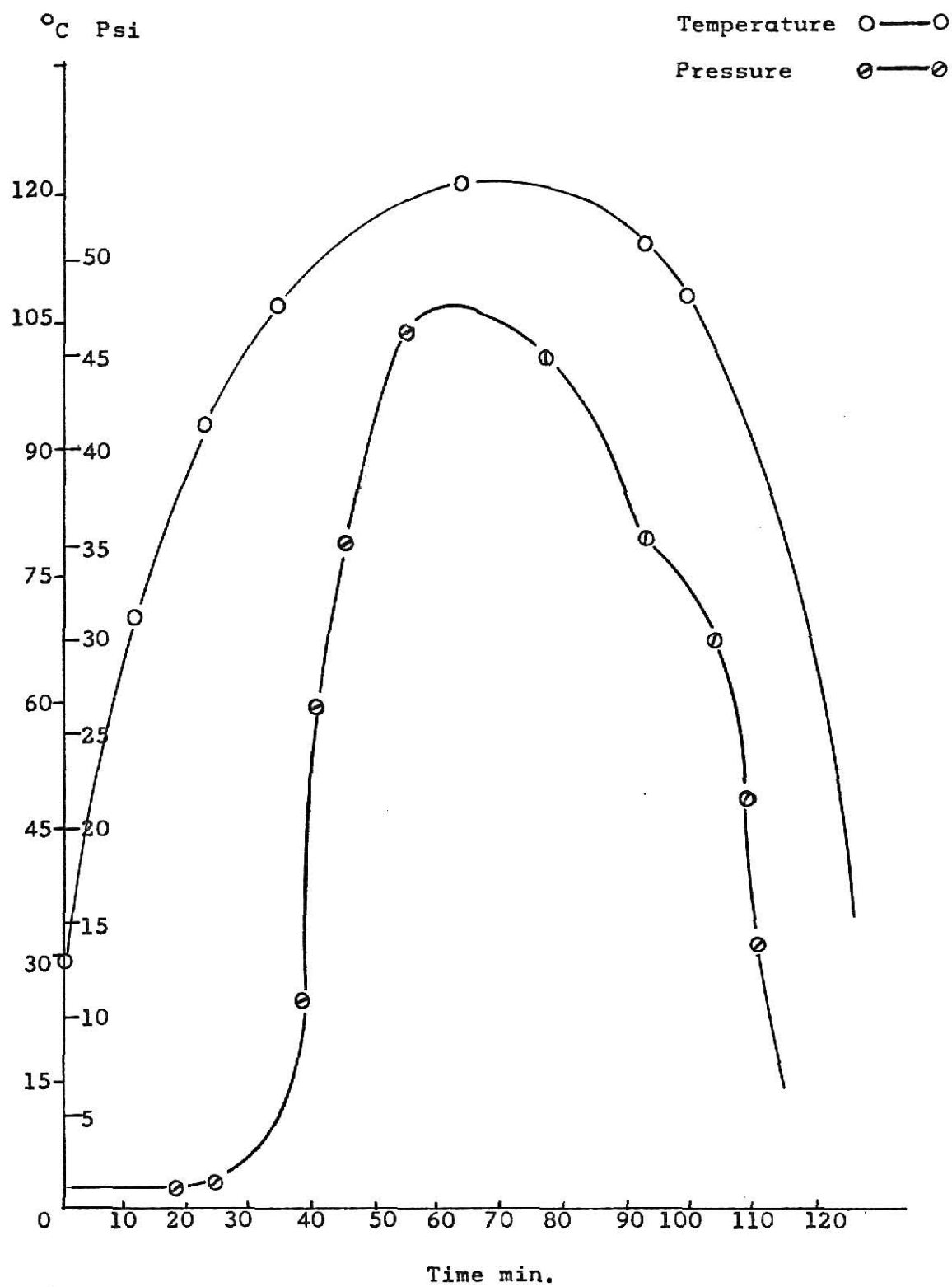


Fig. 9 Changes in temperature and pressure during the reaction of SMP and Ethylene Oxide in DMF with CH_3ONa as catalyst.

ethoxylation of alcoholic hydroxy compound increases with increasing catalyst basicity (87, 88). Alkali metal carbonates are relatively ineffective for alcohols although they have been successful as catalysts for carboxylic acids (87, 89). The addition of ethylene oxide to alcoholic hydroxy compound is catalyzed by alkali metal hydroxides or alkoxides (87, 88, 89). It was found that alkali metal hydroxide produced much more water than alkoxides. Therefore, CH_3ONa was the catalyst of choice. The amount of CH_3ONa was only trace in the small scale preparation. The amount of CH_3ONa used in the large scale preparation was based upon the apparent pH of DMF, which was 11.5 (23), as measured by Beckman pH meter using a glass electrode and calometric reference electrode (after the electrodes were allowed to equilibrate in DMF). The addition of SMP lowered the apparent pH. CH_3ONa returned the pH back to 11.5. Thus, the amount of CH_3ONa used in the preparation was equivalent to the amount needed to bring the apparent pH back to 11.5.

Solvent System. The solvent used in the preparation must be relatively free of water since water will cause the formation of polyethylene glycol as a by-product. The mutual solvent, DMF, was used for SMP since it has a high dielectric constant and high polarities but lacks hydroxy or amino groups. It dissolves SMP which contains hydrophilic and lipophilic groups without charring effect. DMF decomposes slightly at its normal B. P. to give small amounts of dimethylamine and carbon monoxide. The decomposition is catalyzed by alkali. At room temperature, DMF is appreciably decomposed if allowed to stand for a considerable period of time in the presence of potassium hydroxide or sodium hydroxide (90). The percentages of yield in proportion to varying concentrations of SMP, other conditions

being the same, are presented in Table 3.

Table 3

Percentages of Yield on Various Concentrations of SMP in DMF Solvent.

SMP/DMF	20%	10%	5%
Yield	Approx. 50%	Approx. 65-70%	Approx. 40%

The above data were obtained by small scale preparation. SMP/solvent ratio at 10% gave the highest yield. Dimethyl sulfoxide (DMSO) could serve as solvent, too. Several other solvents such as methylene chloride and diethylene glycol were tried, but were considered inadequate.

By-product. The major by-product was polyethylene glycol. During the ethoxylation reaction, the presence of any amount of water in ethylene oxide or SMP or water generated from the catalyst was responsible for the formation of polyethylene glycol. The acidity of water is comparable to the acidity of primary alcohols, consequently, water can compete effectively as an acid (53).



Nucleophilic attack on ethylene oxide by hydroxide ion forms ethylene glycol, which, on further addition of ethylene oxide, gives polyoxyethylene glycol. It should be noted that each glycol molecule contains two hydroxyl groups, effectively doubling its probability of further chain growth. Therefore, ethylene oxide must be at least 99.5% pure and contain no more than 0.03 w/w water. Ethylene oxide

with this specification is available in the market. SMP was recrystallized with methylethylketone and dried over P_2O_5 by reduced pressure overnight to remove any possible moisture. Fresh CH_3ONa were prepared just before the ethoxylation reaction. Based upon the foregoing discussion on starting materials, temperature, catalyst and solvent system, a lowered polyethylene oxide yield could be obtained by careful selection of catalyst, the absence of water, and a lowered reaction temperature.

Reaction Conditions. The reaction conditions for the ethoxylation of SMP were as follows:

- 1) Temperature: 110-120°C
- 2) Pressure: 40-50 psi
- 3) Reaction time: 2-2½ hrs.
- 4) Ethylene oxide flow rate: 1 bubble/2 sec
- 5) SMP/solvent (DMF): 1/10
- 6) Molar ratio (Ethylene oxide/SMP): 20/1
- 7) Catalyst: Small scale preparation- trace amount of CH_3ONa
Large scale preparation- equivalent amount of CH_3ONa that brings the apparent DMF pH after the addition of SMP back to 11.5

Separation and Isolation. The composition of the starting material, SMP, is very complicated. Linow et al. (91) separated 11 different esters spots for a sucrose palmitate preparation on a one-dimensional TLC plate. SMP consists of mono-, di-, and tri-esters of sucrose. The monoesters contain 85-90% sucrose esters, of which 90% is made up of various isomers. Fatty acid components of SMP are stearic and palmitate. Due to the complicated nature of SMP, after

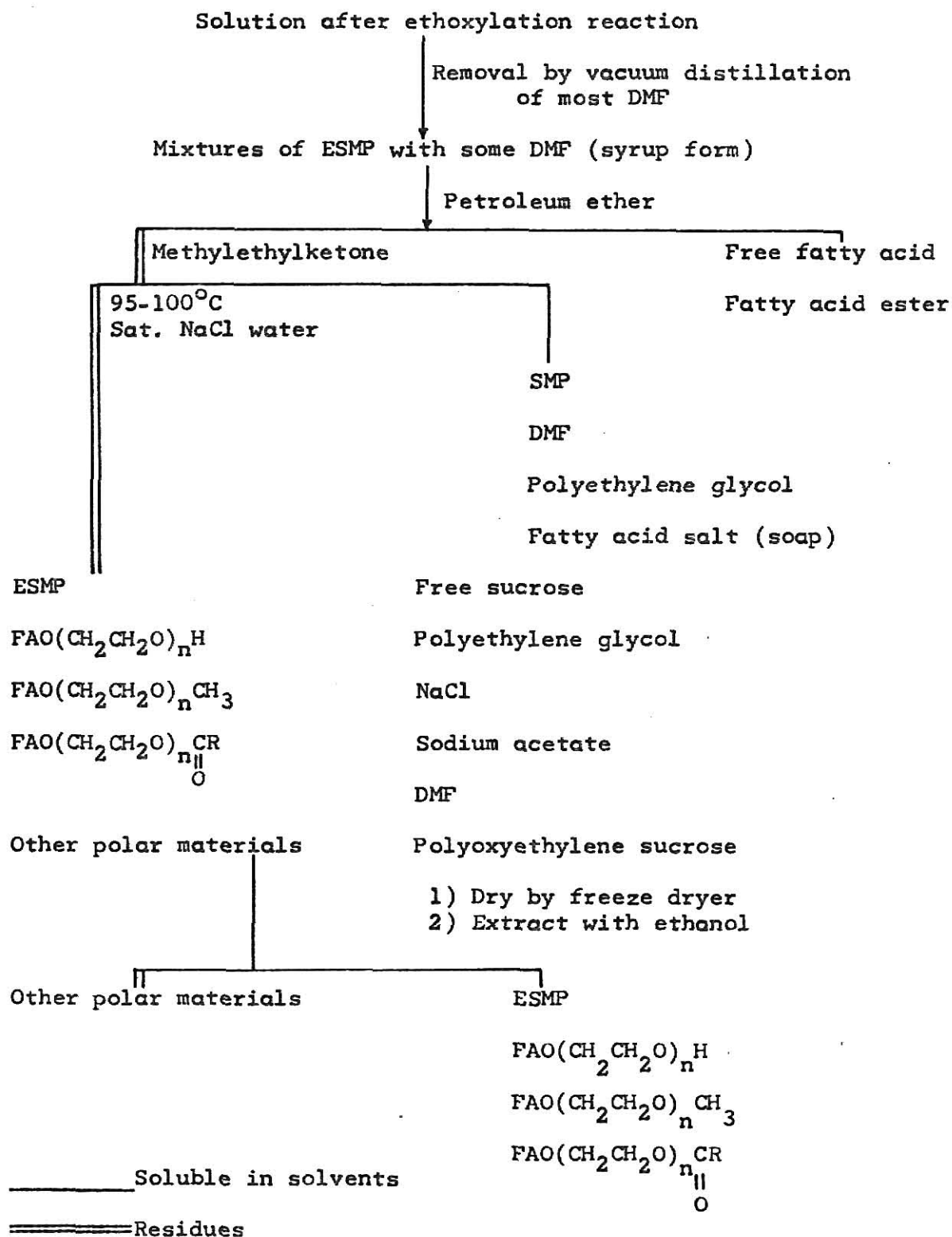
ethoxylation, the crude product contained not only ESMP, but also quite a number of by-products and foreign materials mixed in with ESMP. There could possibly be in the crude product the following compounds:

- 1) ESMP (major product)
- 2) Free fatty acid
- 3) Fatty acid methyl ester
- 4) Free sucrose
- 5) Soaps (salts of fatty acids)
- 6) Dimethyl formamide (solvent)
- 7) Sodium acetate
- 8) Unreacted SMP
- 9) Polyethylene glycols (PEG)
- 10) Polyoxyethylene fatty acid
- 11) Sodium chloride
- 12) Polyoxyethylene sucrose
- 13) Other polar materials

Separation and isolation were difficult since a complicated mixture of by-products and foreign materials were obtained with ESMP. Moreover, the chemical and physical properties of some of these by-products were very similar to those of ESMP. An effort was made to isolate the crude product and remove most of the by-products. Separation of pure ESMP was not achieved. Silica gel column chromatography with chloroform as eluting solvent was tried but failed. Additional research is required to find a separation technique that could successfully result in pure ESMP. A flow chart for the extraction of ethoxylation reaction crude products is presented in Table 4.

Table 4

Crude Product ESMP Fractionation Procedure



Ethanol extraction was only successful to the small scale preparation product ESMP I which was dissolved. It failed in ESMP II which was only soluble in chloroform, probably due to some other unknown impurities. Solubilities were compared among small scale preparation product ESMP I, large scale preparation product ESMP II, and starting material SMP in Table 5.

Table 5
Comparison of Solubilities of ESMP I, ESMP II, and
SMP in Solvent and Water Solution

	Chloroform	Methyl ethyl ether	Acetone	Methanol	Ethanol	Water
ESMP I	S	S	S	S	S	S
ESMP II	S	SS	SI	SI	I	SS
SMP	S	SS	SS	SS	SS	SS

S= Soluble

SS= Slightly soluble

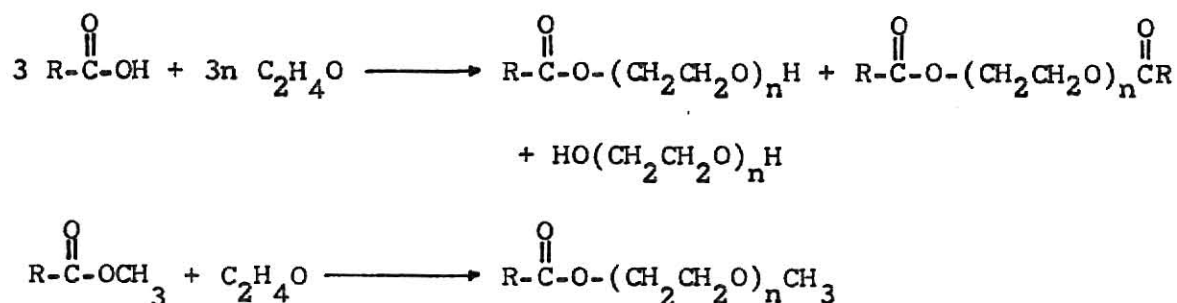
SI= Poor soluble

I= Insoluble

After ethoxylation, the solution was vacuum distilled until it became in the form of a syrup, extracted with petroleum ether (2:1 V/V) twice and then filtered so that free fatty acid and fatty acid ester were removed and monitored by TLC plates which were developed with a mixture of petroleum ether-ether (1:1 V/V). The filtrate was extracted with methylethylketone, removing parts of DMF and any fatty acid salt (soap). The residue after extraction was detected by TLC plate developed with a mixture of acetone-chloro-

form (4:1 V/V) and compared with starting material SMP. No SMP was present in the ethoxylated mixture on the chromatogram.

The high solubility characteristic of polyethylene glycol (PEG) in water suggests that the components of the mixture could be selectively extracted. Monoesters will follow an inverse solubility-temperature relationship in water and will also be less soluble in salt solutions. This behavior is typical of nonionic surfactants. Monoesters are found to be substantially insoluble in hot salt solution where the PEG dissolves and, consequently, can be separated (80). The major by-products, polyethylene glycols were removed by extraction with saturated NaCl water solution at 95-100°C. Evidence of their removal was shown on TLC (ethanol-chloroform (1:3 V/V)) chromatogram which were compared with high (M. W. 3,000-3,700, BDH) and low (M. W. 400, Fisher) molecular weight PEG. Finally, there were still quite a few impurities in the ESMP mixtures. When free fatty acid and fatty acid methyl ester were generated during ethoxylation reaction the following compounds were obtained:



PEG could be removed by saturated sodium chloride water at 95-100°C but $\text{FAO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ and $\text{FAO}(\text{CH}_2\text{CH}_2\text{O})_n\text{COR}$ and maybe some other near polar materials still remained in the mixture.

There are several ways to determine the number of oxyethylene

units in the polyoxyethylene compounds; by saponification, equivalent carbon and hydrogen analysis, iodine number (A), and weight before and after addition of ethylene oxide (B). Ballun et al. (92) showed that all methods give decreasing precision with increasing molecular weight. Method A is recommended for compounds with shorter chains, but method B is preferred in the case of products having a larger number of oxyethylene groups. Since a long oxyethylene chain is expected to be in the ESMP molecule, method B was used (Table 6). One thing should be made clear here, the product still contains a few impurities, therefore, the data are only for reference and are not the definite value of oxyethylene units.

Table 6

Data of Weighing Method for the Determination of
Possible Average Number of Oxyethylene Units

<u>Weight before</u>	<u>Weight after</u>	<u>Possible average no. of oxyethylene units</u>
1.723 g	2.515 g	18

Polyoxyethylene type nonionic surfactants contain oxyethylene units which give an extremely sensitive color reaction with Draggendoff reagent (81). The sensitivity of this reaction increases with the number of mole oxyethylene per molecule. ESMP gave a positive test to form red-orange color when it was treated with Draggendoff reagent. Fatty acid ester bond of ESMP was also checked after acid (6N HCl) hydrolysis and monitored by TLC plates developed in petroleum ether-ether (9:1 V/V) solvent system.

Baking Tests. There are several parameters used in breadmaking to determine the qualities of bread. The most important parameter is loaf volume. Loaf volume is highly correlated, over a relatively wide range, with consumer acceptance, technological versatility, rheological properties, and other important breadmaking characteristics including inherent protein quality, protein content, and oxidation requirement (93). There are adverse effects when soy flour is added to wheat flour, such as the drastic reduction in loaf volume, thick rough crust, coarse grain, open crumb texture and poor symmetry. Of special importance is the non-glutenous protein adjuncts which exert a volume-depressing effect on bread (94). Two series of baking tests were conducted, one using ESMP I and the other using ESMP II. The effect of surfactants on loaf volume and specific loaf volume of bread baked from wheat flour fortified with 12% soy flour is shown in Tables 7 and 8.

ESMP I (Table 7) was successful for making soy fortified bread. The average loaf volume, with 0.25% ESMP I added, was 2412 compared to 2305, 2062, 2225 with the same percentage of SSL, control, and SMP, respectively. The average specific volume was 5.45 for 0.25% ESMP I while 5.15, 4.96, 4.58 were obtained with 0.25% of SSL, SMP, and control, respectively.

Baking experiments with synthetic glycolipids indicated that both hydrophobic and hydrogen bonds are important for the improving effect of glycolipids (95). Glycolipids containing a disaccharide were superior as improvers to corresponding monosaccharide derivatives (93). Pomeranz et al. (4) showed that synthetic glycolipids (of the sucroester types) could be used to counteract the highly deleterious effects on loaf volume of high concentrations of soy

Table 7
Effect of 0.25% ESMP I, SMP, SSL, and Control on Loaf Volume
and Specific Loaf Volume of Bread Baked from Wheat Flour
With the Addition of 12% Soy Flour

Surfactant*	Loaf weight (g)	Loaf volume (c. c.)	Sp. loaf vol. (c. c. /g.)	Avg. sp. loaf vol. (c. c. /g.)
Control	435	447	2050	4.58
SSL	446	447	2325	5.15
SMP	444	453	2250	4.96
ESMP I	440	445	2425	5.45

* % on flour basis.

flour fortified in nutritionally improved bread. The effects of synthetic glycolipids increase with an increase in the degree of hydrophilicity.

ESMP I was much more soluble in high polar solvents: methanol, ethanol, and even in water than SMP. Releasing the "glue" effect in ESMP molecule by adding oxyethylene group will probably make it more soluble in water. The zig-zag from of long oxyethylene chain attached to the ESMP molecule provide more hydrogen bonding sites which are the lone pair electron on oxygen atom of oxyethylene group to interact with the hydrogen atom of amino group of wheat protein and soy protein.

Conversely, ESMP II failed in another series of soy fortified baking tests (Table 8). The specific volumes of soy fortified bread of 0.25% and 0.5% ESMP II were only 5.25 and 5.50 compared with those of SMP, SSL, and control of 6.12 and 6.40, 5.53 and 6.38, and 4.73. SMP showed not only better specific loaf volume than SSL but also much better than ESMP II. In the earlier series of baking tests using ESMP I, it was found to be the best, with SSL next and then SMP. There were several factors which might possibly have caused the negative results obtained by ESMP II:

- 1) Starting material SMP was of a different quality in the small and large scale preparations.
- 2) The number of average oxyethylene groups attached to SMP molecule was not 20 units in the large scale preparation of ESMP.
- 3) Conditions obtained in the small scale preparation of ESMP in the resin kettle which when applied to the large scale preparation would perhaps require some modifications with the use of the high pressure bomb.

Table 8

Effect of 0.25% and 0.5% ESMP II, SMP, SSL, and Control on Loaf
Volume and Specific Loaf Volume of Bread Baked from Wheat Flour
With the Addition of 12% Soy Flour.

Surfactant*	Loaf weight (g)	Loaf volume (c. c.)	Sp. loaf vol. (c. c. /g.)	Avg. sp. loaf vol. (c. c. /g.)			
Control	488	447	2075	2150	4.63	4.81	4.72
SMP 0.25%	433		2650		6.12		6.12
SMP 0.5%	431	440	2800	2725	6.50	6.31	6.40
ESMP II 0.25%	443	443	2350	2300	5.30	5.29	5.25
ESMP II 0.5%	436	441	2400	2425	5.50	5.50	5.50
SSL 0.25%	440	441	2425	2450	5.51	5.55	5.53
SSL 0.5%	432	441	2750	2825	6.37	6.40	6.38

*% on flour basis.

- 4) Isolation and purification technique problems.
- 5) Retransesterification could be happening during large scale preparation to form more percentages of di-, tri-, tetra-esters and make the final product more lipophilic.

Effect of ESMP on Dough Properties. It has been demonstrated by means of the farinograph and extensigraph that various percentages of surfactants exhibit different effects on the rheological properties of wheat flour-water dough with or without the addition of soy flour and such differences can hardly be correlated with the improving effects of surfactants on specific loaf volume and quality characteristics of the resulting loaves (96). Although the above statement is true, the various percentages of large scale preparation product ESMP II were tried by farinograph and extensigraph to see what the effects were.

Farinograph. The farinogram data shown on Table 9 were obtained from wheat flour, wheat flour containing various percentages of surfactants (0.25-1.0%), wheat flour mixed with soy flour (12%), and wheat flour containing various percentages of surfactants (0.25-1.0%) and soy flour (12%).

Water absorption of the wheat flour water-dough was 62.5%. With the addition of surfactant to wheat flour, water absorption varied only slightly between 62.5-60.8%. Addition of soy flour to the wheat flour increased water absorption considerably, from 62.5% (control wheat flour) to 67.5%. Addition of ESMP II and SMP brought about only minor changes of water absorption.

It would be expected that polyoxyethylene surfactant would increase water absorption by wetting effect, however, evidence shows no correlation between increased water absorption and the

Table 9
 Characteristics of Farinograms of Doughs Prepared From Wheat Flour
 With Soy Flour and/or Surfactants Added

Surfactants*	Wheat Flour					Wheat Flour, 12% Soy Added				
	Absorp- tion %	Arrival time min.	Peak time min.	Dough stability min.	M.T.1 B.U.	Absorp- tion %	Arrival time min.	Peak time min.	Dough stability min.	M.T.1 B.U.
Control	62.5	2.5	8.0	9.5	15	67.5	5.0	7.5	7.5	20
SMP 0.25%	62.8	3.5	7.5	11.5	20	67.5	4.5	7.5	9.0	20
SMP 0.5%	62.5	3.0	7.5	12.5	20	68.2	4.5	7.5	9.0	20
SMP 1.0%	62.5	3.0	6.5	13.5	15	68.2	4.5	7.5	9.0	20
ESMP II 0.25%	62.0	4.0	6.5	8.0	20	67.5	5.5	7.5	7.0	20
ESMP II 0.5%	61.5	2.5	6.0	12.0	25	67.5	5.0	7.5	8.5	20
ESMP II 1.0%	61.5	3.5	6.5	12.5	25	67.5	5.0	7.5	10.0	20

* % on flour basis.

functional characteristics of these surfactants, as measured by their effects on loaf volume (96). ESMP II was not found to increase water absorption more than the starting material SMP and even decreased it slightly. It could be caused by impurities and probably retransesterification happening during ethoxylation reaction caused the formation of more multiple esters such as tri-, and tetra-esters.

For the control wheat flour dough, the arrival time was 2.5 min. When various percentages of SMP were added during mixing, the arrival time varied from 3.5-3.0 min. When various levels of ESMP II were added, it varied from 2.5-4.0 min. Addition of soy flour to control flour increased the arrival time to 5 min. 4.5 min. was the only arrival time obtained with various levels of SMP. It varied from 4.0-4.5 min. with the addition of various percentages of ESMP II.

The arrival time of the farinograms could be related to the rate of hydration of the gluten protein. Therefore, it reflected the structural changes in the gluten protein during mixing with ESMP II and /or SMP.

Peak time of the farinograph shows optimum dough development. In the wheat flour series, peak time for the control was 8 min., 7.5-6.5 min. for various percentages of SMP, and 6.0-6.5 min. for various percentages of ESMP II. On the other hand, the addition of 12% soy flour to wheat flour changed the peak time to 7.5 min. Various percentages of SMP and ESMP II all showed no change in peak time.

The strength of gluten structure during mixing is reflected by dough stability (97). Dough stability of control wheat flour was 9.5 min., decreasing to 7.5 min. when 12% soy flour was added. 0.25-1.0% of SMP added to control wheat flour changed dough stability from 11.5 to 13.5 min. There was a decrease to 8 min. when 0.25%

ESMP II was added but an increase to 12 and 12.5 min., when 0.5% and 1.0% ESMP II were added, respectively. When various percentages of SMP were added in wheat flour with 12% soy flour, dough stability in each was 9 min. 0.25% ESMP II decreased dough stability to 7 min., while 0.5% and 1.0% ESMP II increased it to 8.5 and 10.0 min., respectively. Thus, the large scale preparation product ESMP II showed inferior results with regards to dough stability in comparison with SMP in wheat flour and in wheat flour with 12% soy flour.

Extensigraph. At 45 min. resting time, the (R_{50}) resistance at 50 mm extensibility was 160 B. U. For the control wheat flour-water dough (Table 10), its maximum resistance (R_m) and extensibility (E_x) were 175 B. U. and 130 mm, respectively. When 0.25% and 0.5% SMP were added, R_{50} changed to 140 and 150 mm. R_{50} for 0.25% and 0.5% ESMP II were about the same as those for SMP, whereas the R_m 's were quite different. 0.25% ESMP II had a R_m of 195 B. U. which was lower than that for SMP, while 0.5% ESMP II had 290 B. U., much higher than that for SMP. E_x for 0.25% ESMP II was 184 B. U. which was the highest obtained for the various percentages of ESMP II and SMP. E_x ranged between 140-184 for 0.25% to 1.0% ESMP II and SMP.

After 90 min., R_{50} , R_m , and E_x increased to 170 B. U., 200 B. U. and 153 mm, respectively. When the dough contained surfactant, R_{50} ranged between 200-250 B. U., while R_m ranged between 200-335 B. U. For E_x , only the 0.25% SMP decreased to 150 mm and the other levels of ESMP II and SMP had increased E_x .

In general, R_m for extension of all the various percentages of surfactants tended to increase as dough was rested from 45 to 90 min.

12% soy flour was added to wheat flour and changed R_{50} and

Table 10
 Characteristics of Extensigrams of Dough Prepared From
 Wheat Flour and Surfactants.

Surfactants *	Resting time					
	45 min.			90 min.		
	R ₅₀ ^a	R _m ^b	E _x ^c	R ₅₀	R _m	E _x
	B. U.	B. U.	mm.	B. U.	B. U.	mm.
Control	160	175	130	170	200	153
SMP 0.25%	200	210	140	200	230	150
SMP 0.5%	210	225	150	220	235	160
ESMP II 0.25%	200	195	184	222	200	185
ESMP II 0.5%	210	290	165	250	335	170

* % on flour basis.

^a Resistance to extension at 50 mm. extensibility.

^b Maximum resistance to extension.

^c Extensibility.

R_m drastically from between 160-200 to 375-550. Extensibility changed from between 130-153 to 105-110 (Table 11). Extensigraph procedures determine simultaneous changes in dough properties during fermentation. The protein structure formed during mixing is determined by farinograph and further developed by fermentation, punching, and molding. The ability of gluten protein to form thin film is related to gas retention. A dough must be sufficiently rigid to prevent its rapid spreading and influence of gravity or accumulation of gas cells at the top. Moreover, if it is too stiff, gas cells cannot expand during proofing, or their expansion is accompanied by rupture of the membrane between them which leads to a coarse crumb (98). Therefore, gas retention depends on a balance of properties that are required for resistance and extensibility, i. e., the ratio E_x/R_m . Comparing the results when SMP or ESMP II were added, the ratio of E_x/R_m was higher in SMP than in ESMP II. Since the change of extensibility can be characterized by the ratio E_x/R_m , and could reflect the ability of gluten protein to retain gas, therefore, SMP is better than ESMP II in this respect.

Suggestions for Further Research. The most difficult work will be in obtaining a qualitatively and quantitatively purified ESMP. The conditions for large scale preparation require further study. Once purified, structure identification and true average number of oxyethylene unit per molecule will be obtained. The determination of hydroxy number, saponification number and iodine number make possible the calculation of hydrophilic lipophilic balance (HLB) which is a very important characteristic of nonionic surfactants. Spectroscopic methods such as UV (99, 100), IR (84), and NMR (101) can also be used for the identification of the mole-

Table 11
 Characteristics of Extensigrams of Wheat Flour-Water Doughs
 Containing Soy Flour and Surfactants.

Surfactants [*]	Resting time					
	45 min.			90 min.		
	R ₅₀ ^a	R _m ^b	E _x ^c	R ₅₀	R _m	E _x
	B. U.	B. U.	mm.	B. U.	B. U.	mm.
Control	375	400	110	490	550	105
SMP 0.25%	330	360	123	520	575	108
SMP 0.5%	375	400	116	550	600	102
ESMP II 0.25%	370	400	115	565	622	105
ESMP II 0.5%	368	430	130	563	610	105

^{*} % on flour basis.

^a Resistance to extension at 50 mm. extensibility.

^b Maximum resistance to extension.

^c Extensibility.

cular structure. For reasons of physiological safety, the average number of oxyethylene units should be over 6-7 (102) and DMF should not be over 0.1% in ESMP (103). Infrared analysis can be successfully employed in the determination of very small quantities of DMF (103). A new method of preparing ethoxylated sucrose esters without solvent system is being researched in Europe at the present time. The process consists of reacting ethylene oxide with sucrose in suspension in a fatty acid ester, in the presence of potassium hydroxide as catalyst (67).

CONCLUSION

Desirability of bread as a vehicle for protein fortification is reflected by the fact that it is a staple food in many areas of the world. It is a challenging area for cereal chemists and technologists to improve the quality of high-protein bread to meet consumer requirements with respect to loaf volume, texture, symmetry, and grain.

Due to the high strain energy of ethylene oxide, it could be polymerized with alcoholic hydroxy group on the SMP molecule to form ESMP. A possible explanation of the mechanism of the improving action of ESMP in making soy fortified bread could be derived from the assumption of its releasing binding effect of water molecules between polyhydroxyl groups of SMP and providing more hydrogen bonding sites enabling interaction of both proteins of wheat and soy, making high-protein bread.

Comparing the specific loaf volumes with the use of relatively pure ESMP I (0.25%) with the same amount of SMP, SSL, and control in a series of baking tests using wheat flour with 12%

soy flour added, it was found to be greatest with ESMP I.

There is evidence that ESMP is a high potential surfactant for making high-protein bread. Although ESMP I was relatively successful in making high-protein bread, when proceeding to the large scale preparation (ESMP II), it was shown through the baking test, farinograph and extensigraph to be a failure.

Intensive research should be furthered in the large scale preparation, isolation, purification, structural identification, improvement mechanism and physiological effects, which largely involve organic, analytical, physical, and polymer chemistry, and physiology. Once these can be accomplished, it will be a useful surfactant for high-protein breadmaking and for solving protein malnutrition.

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LITERATURE CITED

1. Altschul, A. M. A look at the world protein situation. Soybean Dig. 26(8):15 (1966).
2. Tsen, C. C., and Tang, R. T. K-State process for making high-protein breads. I. Soy flour bread. Baker's Dig. 45(5):26 (1971).
3. Tsen, C. C., Hoover, W. J., and Phillips, D. High-protein breads. Use of sodium stearyl-2 lactylate and calcium stearyl-2 lactylate in their production. Baker's Dig. 45(2):20 (1971).
4. Pomeranz, Y., Shogren, M. D., and Finney, K. F. Improving bread-making properties with glycolipids. I. Improving soy products with sucroesters. Cereal Chem. 46:503 (1969).
5. Pomeranz, Y., Shogren, M. D., and Finney, K. F. Improving bread-making properties with glycolipids. II. Improving various protein-enriched products. Cereal Chem. 46:512 (1969).
6. Patco Products: Emplex, Verv- Their use and function in foods. Technical Bull. C. J. Patterson Co., Kansas City, Missouri.
7. Atlas Products: General characteristics of Atlas surfactants. Technical Bull. Atlas Chemical Industries, Inc., Wilmington, Delaware.
8. Ponte, J. G., Jr. Bread. In: Wheat Chemistry and Technology. ed. Pomeranz, Y. p.715. Am. Assocn. Cereal Chem., St. Paul, Minn. (1971).
9. Tsen, C. C., and Hoover, W. J. The shortening-sparing effect of sodium stearyl-2 lactylate and calcium stearyl-2 lactylate in bread baking. Baker's Dig. 45(3):38 (1971).
10. Dubois, D. K., and Ash, D. J. New dough conditioning/softener systems. Baker's Dig. 45(1):32 (1971).
11. Hass, H. B. Early history of sucrose esters. Sugar Research Foundation, Inc.
12. Berthelot, M. Sur les combinaisons des sucres avec les acides on saccharids. Ann. Chim. Et. Phys. 60:93 (1860).
13. Hess, K., and Hessmer, E. Synthesis of fatty acid derivatives of sugars I. Chem. Ber. 54B, 499-523 (1921). C. A. 15:2422 (1921).
14. Harris, B. R. U. S. Patent 1,917,250 (July 11,1933); Ibid., 1,917,257 (July 11,1933).
15. Lorand, E. J. U. S. Patent 1,959,590 (May 22,1934).
16. Zief, M. Unsaturated esters of sucrose. J. Amer. Chem. Soc. 72:1137 (1950).

17. Osipow, L., Snell, F. D., York, W. C., and Finchler, A. Methods of preparation: Fatty acid esters of sucrose. *Industr. Engng. Chem.* 48(9):1459 (1956).
18. O'Boyle, C. J. Sucrose esters. In: *Proceedings of the first international sugar research conference, Brussels, Belgium, March 10-11 (1970).*
19. Osipow, L. I., and Rosenblatt, W. Micro-emulsion process for the preparation of sucrose esters. *J. Amer. Oil Chemists' Soc.* 44:307 (1967).
20. Curtis, G. W. U. S. Patent 2,999,858 (1961).
21. D'Amato, V. (Ledoga S. p. A.) U. S. Patent 3,054,789 (1962).
22. Feuge, R. O., Zeringue, H. J., Jr., Weiss, T. J., and Brown, M. Preparation of sucrose esters by interesterification. *J. Amer. Oil Chemists' Soc.* 47(2):56 (1970).
23. Sugar Research Foundation, Inc., "Sucrose Ester Surfactants," New York (1960).
24. York, W. C., Finchler, A., Osipow, L., and Snell, F. D. Structural studies on sucrose monolaurate. *J. Amer. Oil Chemists' Soc.* 33: 424 (1956).
25. Bonner, W. A. C1-C2 Acetyl migration on methylation of the anomeric 1, 3, 4, 6-tetra-O-acetyl-D-glucopyranoses. *J. Org. Chem.* 24:1388 (1959).
26. Lemieux, R. U., and McInnes, A. G. The preparation of sucrose monoesters. *Can. J. Chem.* 40:2376 (1962).
27. Lemieux, R. U., and McInnes, A. G. The composition of the sucrose monomyristate prepared by transesterification. *Can. J. Chem.* 40:2394 (1961).
28. Reinefeld, E. Selective acylation of sucrose: Isolation and structure of partial esters of fatty acids. *Klaudianos* 21(12): 330 (1968).
29. Otake, T., and Tamate, E. Isomers of sucrose monopalmitates I, II. *Nippon Kagaku Zasshi* 90(4):388,393 (1969). *C. C.* 71:30643V (1969).
30. Wurtz, A. Memoire sur les glycols on alcohols diatomiques. *Ann. Chim. Et. Phys.* 55:400 (1859).
31. Schultze, H. C. In: *Glycols*. eds. Curme, G. O., Jr., and Johnston, F. chap. 5. Reinhold, New York (1952).
32. St. Pierre, L. E. In: *Polyethers, Part 1, Polyalkylene Oxide*. ed. Gaylord, N. G. chap 3. Wiley-Interscience, New York (1963).

33. Winstein, S., and Henderson, R. B., *Heterocyclic Compounds*, Vol. I. ed. Elderfield, R. C. Wiley, New York (1959), p.1.
34. American Conference of Governmental Industrial Hygienists, *Arch. Environ. Health* 9:545 (1964).
35. Ingold, C. K. *Structure and Mechanism in Organic Chemistry*. p. 341. Cornell Univ. Press, Ithaca, New York (1953).
36. Parker, R. E., and Isaacs, N. S. Mechanisms of epoxide reactions. *Chem. Rev.* 59:737 (1959).
37. Cox, J. D. Strain energies of cyclic compounds. *Tetrahedron* 19:1175 (1963).
38. Cunningham, G. L., Boyd, A. W., Myers, R. J., Gwinn, W. D., and Leven, W. I. The microwave spectra, structure, and dipole moments of ethylene oxide and ethylene sulfide. *J. Chem. Phys.* 19:676 (1951).
39. Turner, T. E., and Howe, J. A. Determination of molecular structures from rotational spectra measurements. *J. Chem. Phys.* 24:924 (1956).
40. Pauling, L. *The Nature of the Chemical Bond*. p. 112. 3rd ed., Cornell Univ. Press, Ithaca, New York (1960).
41. Coulson, C. A., and Moffitt, W. E. Strain in non-tetrahedral carbon atoms. *J. Chem. Phys.* 15:151 (1947).
42. Coulson, C. A., and Moffitt, W. E. Properties of certain strained hydrocarbons. *Phil. Mag.* 40:1 (1949).
43. Burwell, R. L. The cleavage of ethers. *Chem. Rev.* 54:615 (1954).
44. Sorenson, W. R., and Campbell, T. W. *Preparative Methods of Polymer Chemistry*. p. 342. 2nd ed., Interscience Publishers, New York (1968).
45. Shachat, N., and Greenwald, H. S. Mechanism of Ethylene Oxide Condensation in Nonionic Surfactant. p. 11. ed. Schick, M. J., Marcel Dekker, Inc., N. Y. (1967).
46. Gee, G., Higginson, W. C. E., Levesley, P., and Taylor, K. J. Polymerization of epoxides. Part I. Some kinetic aspects of the addition of alcohols to epoxides catalyzed by sodium alkoxides. *J. Chem. Soc.* 1338 (1959).
47. Stockburger, G. J., and Brandner, J. D. The reactions of alkylene oxides with various butyl and other alcohols. *J. Amer. Oil Chemists' Soc.* 40:590 (1963).
48. Weibull, B., and Nycander, B. The distribution of compounds formed in the reaction between ethylene oxide and water, ethanol,

ethylene, glycol, or ethylene glycol monoethyl ether. *Acta Chem. Scand.* 8:847 (1954).

49. Nagase, K., and Sakaguchi, K. Distribution of the degree of polymerization of condensation products of ethylene oxide with lauryl alcohol and nonyl phenol. *Kogyo Kagaku Zasshi* 63:588 (1960). *C. A.* 56:4929h (1962).
50. Lowe, A. J., and Weibull, B. Zur frage der anion polymerisation. *J. Polymer Sci.* 12:493 (1954).
51. Gee, G., Higginson, W. C. E., and Merrall, G. T. Polymerization of epoxides. Part II. The polymerization of ethylene oxide by sodium alkoxides. *J. Chem. Soc.* 1345 (1959).
52. Dainton, F. S., Devlin, T. R. E., and Small, P. A. The thermodynamics of polymerization of cyclic compounds by ring opening. Part I. *Trans. Faraday Soc.* 51:1710 (1955).
53. Schonfeldt, N. Surface Active Ethylene Oxide Adducts. p. 102. Pergamon Press, New York (1969).
54. Schoeller, C., and Wittwer, M. U. S. Patent 1,970,578 (1934).
55. Griffin, W. C. U. S. Patent 2,374,931 (1945).
56. Griffin, W. C. U. S. Patent 2,380,166 (1945).
57. Iino, S. Studies on the synthesis of polyoxyethylene sorbitan fatty acid esters. I. Discussions of their constitutions from analytical data and synthesis of them. *J. Jap. Oil Chemists' Soc.* 10(7):427 (1961).
58. Gluzman, M. K., Dashevskaya, B. I., and Fridman, G. M. Preparation of monoesters of sorbitan (speres) and of products of their oxyethylation (tweens). *Zh. Prikl. Khim.* 38(10):2319 (1965).
59. I. G. Farbenindustrie A. G., British Patent 375:842 (1932).
60. Schmidt, O., and Meyer, E. U. S. Patent 1,959,930 (1934).
61. Tahan, M., and Zilkha, A. Anionic graft polymerization of ethylene oxide on starch. I. *J. Polym. Sci. A-1* 7:1815 (1969).
62. Tahan, M., and Zilkha, A. Anionic graft polymerization of ethylene oxide on starch. II. Structure of the graft polymers. *J. Polym. Sci. A-1* 7:1825 (1969).
63. Ezra, G., and Zilkha, A. Grafting of preformed polyethylene oxide on starch. *J. Appl. Polym. Sci.* 13:1493 (1969).
64. Ezra, G., and Zilkha, A. Grafting of preformed polyethylene oxide on dialdehyde starch by acetal formation. *J. Macromol. Sci.-Chem.*

A4 (4):957 (1970).

65. LeMaistre, J. W., and Seymour, R. B. The reaction of sucrose with ethylene oxide. *J. Org. Chem.* 13:782 (1948).
66. Reeder, J. A., Rayner, H. B., Aitken, G., Bradley, D., and Atkinson, J. Reactions of sodium sucrate in solution: Preparation of pure long chain ethers of sucrose. I and EC Product Research and Development 7:230 (1968).
67. Duchateau, G. Sugar based surfactants. In: Proceedings of the first international sugar research conference, Brussels, Belgium, March 10-11 (1970).
68. MacDonald, I. A. Emulsifiers: Processing and quality control. *J. Amer. Oil Chemists' Soc.* 45(10):584 A (1968).
69. Pollock, J. M., and Geddes, W. F. Soy flour as a white bread ingredient. I. Preparation of raw and heat-treated soy flours, and effects on dough and bread. *Cereal Chem.* 37:19 (1960).
70. Endres, J. G. Emulsifiers: A review of their applications in baking. *Baker's Dig.* 41(5):96 (1967).
71. Becher, P. Emulsions: Theory and Practice. 2nd ed. p. 222. Reinhold Pub. Corp., New York (1965).
72. Langhans, R. K., and Thalheimer, W. G. Polysorbate 60: Effects in bread. *Cereal Chem.* 48:283 (1971).
73. MacDonald, I. A. Food emulsifiers: Toward a more systematic approach to their selection, development and evaluation. *Baker's Dig.* 38(1):56 (1964).
74. MacDonald, I. A. The functional properties of various surface-active agents. *Baker's Dig.* 42(2):24 (1968).
75. Knightly, W. H., and Lynch, M. J. The role of surfactants in baked foods. *Baker's Dig.* 40(1):28 (1966).
76. Sullivan, B. The reaction of flour proteins with surface-active compounds. *Cereal Chem.* 29:282 (1952).
77. Dai-Nippon Sugar Manufacturing Co., Ltd. Technical information of nittoester. The Company, Chiyoda-Ke, Tokyo, Japan.
78. Hess, L. G., and Tilton, V. V. Ethylene oxide-Hazards and methods of handling. *Industr. Engng. Chem.* 42:1251 (1950).
79. Ferrari, H. J., and Heider, J. G. Purification of dimethylformamide for use in aqueous and nonaqueous microtitrations. *Microchem. J.* 7:194 (1963).
80. Malkemus, J. D., and Swan, J. D. Analysis of polyethylene glycol esters. *J. Amer. Oil Chemists' Soc.* 34:342 (1957).

81. Selden, G. L., and Benedict, J. H. Identification of polyoxyalkylene-type nonionic surfactants by paper chromatography. *J. Amer. Oil Chemists' Soc.* 45:652 (1968).
82. American Association of Cereal Chemists. AACC approved methods. St. Paul, Minn. (1968).
83. Weissberger, A., ed. Heterocyclic Compound With Three-and Four-Membered Rings. Part I and II. Interscience Publishers, New York (1964).
84. Hummel, D. Identification and Analysis of Surface-Active Agents by Infrared and Chemical Methods. Interscience Publishers, New York (1962).
85. Bragg, P. D., and Jones, J. K. N. The characterization of tri-*o*-tosyl sucrose. *Can. J. Chem.* 37:575 (1959).
86. Ethylene oxide. Technical information. Jefferson Chemical Co., Inc., Houston, Texas (1955).
87. Satkowski, W. D., and Hsu, C. G. Polyoxyethylation of alcohols. *Industr. Engng. Chem.* 49:1875 (1957).
88. Sakai, S., and Ishii, Y. Effect of acidity and basicity of catalysts on the rate of ethylene oxide condensation. *Kogyo Kagaku Zasshi* 61:1473 (1958). *C. A.* 56:13088 (1962).
89. Sakakibara, S., Jujuwara, A., and Komori, S. Reaction of higher fatty amines with ethylene oxide. *Kogyo Kagaku Zasshi* 59:1149 (1956). *C. A.* 52:14532 (1958).
90. Stewart, R. The Investigation of Organic Reactions. p. 19. Prentice-Hall, Inc., Englewood Cliffs, New York (1966).
91. Linow, F. H., Ruttloff, and Taufed, K. S. *Naturwissenschaften* 50:689 (1963).
92. Ballun, A. T. Comparative analyses of polyethoxy tallates. *Trans Ill. State Acad. Sci.* 47:81 (1955). *C. A.* 49:164756 (1955).
93. Pomeranz, Y., Finney, K. F., and Hoseney, R. C. Molecular approach to breadmaking. *Science* 167:944 (1970).
94. Marnett, L. F., Tenney, R. J., and Barry, V. D. Methods of producing soy fortified breads. *Cereal Sci. Today* 18(2):38 (1973).
95. Pomeranz, Y., and Wehrli, H. P. Synthetic glycosylglycerides in breadmaking. *Food Technol.* 23:1213 (1969).
96. Aidoo, E. S. High-protein bread: Interactions of wheat proteins and soy proteins with surfactants in doughs and in model systems. Ph. D. dissertation, Kansas State University, Manhattan, Kansas (1972).

97. Bloksma, A. H. Rheology in elucidating functionality in bread-making. Symposium: Composition and Functionality in Breadmaking. A. A. C. C. 55th Annual Meeting. Oct. 18-22, Minneapolis, Minn. (1970).
98. Bloksma, A. H. Rheology and Chemistry of Dough. In: Wheat Chemistry and Technology. ed. Pomeranz, Y. p. 523. Am. Assocn. Cereal Chem., St. Paul, Minn. (1971).
99. Stevenson, D. G. The absorptiometric determination of a nonionic detergent. Analyst 79:504 (1954).
100. Gatewood, L., and Graham, H. D. A spectrophotometric method for the detection and quantitative determination of polyoxyethylene surface-active agents. Anal. Chem. 33(10):1393 (1961).
101. Page, T. F., Jr., and Bresler, W. E. End-group analysis and number-average molecular weight determination of some polyalkylene glycols and glycol polyesters using nuclear magnetic resonance spectroscopy. Anal. Chem. 36(10):1981 (1964).
102. Analytical standard for polyoxyethylene type food additives. Food and Drug Administration, Washington, D. C.
103. Mieth, G., and Linow, F. The use and analysis of saccharose fatty acid esters. Zietschrift fur die Zuckerindustrie 10:528 (1965).

INVESTIGATION ON THE PREPARATION OF ETHOXYLATED
SUCROSE MONOPALMITATE AND EVALUATION OF ITS
POTENTIAL USE FOR MAKING HIGH-PROTEIN BREAD

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The investigation was conducted to prepare ethoxylated sucrose monopalmitate and to evaluate its effects on the rheological properties and baking performance of soy fortified flour.

A. Preparation of ethoxylated sucrose monopalmitate:

Sucrose monopalmitate (SMP) was ethoxylated by addition polymerization of ethylene oxide with dimethyl formamide (DMF) as solvent to form long chain polyoxyethylene derivatives in a resin kettle (for small scale preparation) or a high pressure bomb (for large scale preparation). Ethylene oxide was introduced to the reaction vessel containing SMP either by driving a large quantity of ethylene oxide (in the forms of liquid and gas) by nitrogen gas at room temperature, or by direct addition of a small amount of ethylene oxide at 0°C. Sodium methoxide was used as the catalyst. The molar ratio of ethylene oxide to SMP was 20:1 for the ethoxylation. The temperature and pressure achieved during ethoxylation was dependent on the amount of ethylene oxide added. In this work, the temperature was about $110 \pm 5^{\circ}\text{C}$ and pressure was about 45 ± 5 psi.

After the reaction, the catalyst was neutralized by acetic acid. Most DMF was removed by vacuum distillation. The rest of the syrup form was purified by a series of extractions, especially with saturated sodium chloride water solution which was the best way to remove the major by-product polyethylene glycols (PEG). The removal of PEG from the reaction mixture was checked by thin-layer chromatograms with comparing known high and low molecular weights PEG. Draggendoff reagent (bismuth subnitrate and potassium iodide in acetic acid) gave a red-orange color test for oxyethylene groups and a weighing method was used in estimating the average number of oxyethylene units in

ethoxylated sucrose monopalmitate (ESMP) molecules. The partially purified ESMP still contained a mixture of several components.

B. Effects of ESMP on the rheological properties and baking performance of soy fortified flour:

The crude product, ESMP, obtained from the small scale preparation was compared with sodium stearoyl-2 lactylate (SSL) and SMP (the starting material) by baking tests. Results showed ESMP added at 0.25%, outperformed SMP and SSL in improving the loaf volume of high-protein bread prepared from wheat flour fortified with 12% defatted soy flour.

However, the test with the crude product of ESMP from the large scale preparation failed to support the above results. This suggests that additional studies are required to purify the product and to evaluate again its improving action. The failure could be due to the impurities in the crude product. The effect of the crude product of ESMP on dough properties was examined by farinograph and extensigraph in this investigation.