RAPESEED LECITHIN HYDROXYLATION BY CHLORINE REPLACING WITH HYDROXYL GROUPS IN CHLORINATED PHOSPHOLIPIDS

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Abstract: Rapeseed lecithin ethanol soluble fraction (LESF) was hydroxylated with 30% hydrogen peroxide in the presence of acetic acid. The product was compared to the one obtained by method based on nucleophilic substitution reaction of phospholipids chlorine derivatives. In this approach, hydrogen chloride was added to double bonds in unsaturated acyl groups of phospholipids. Next, chlorine was replaced with hydroxyl groups via the alkaline hydrolysis of chlorine derivatives. The surface active properties of the products obtained with the usage of two methods of rapeseed LESF hydroxylation were determined. The minimal surface tension \( \eta_{\text{min}}, \) mN/m and the critical micelle concentration (CMC, g/L) of LESF hydroxylated with hydrogen peroxide (20.2 mN/m, 6.0 g/L) and obtained by chlorine replacing with hydroxyl groups in chlorinated phospholipids (25.0 mN/m, 9.8 g/L) were compared to LESF (31.8 mN/m, 17.8 g/L). Hydroxylated LESF obtained by lecithin chlorination and chlorine replacing with hydroxyl groups in the chlorine derivatives has no peroxides and the good surface active properties. The product as an effective emulsifier can be used in pharmacy and cosmetics.

Keywords: hydroxylated lecithin, emulsifiers, lecithin modification, phospholipids, rapeseed lecithin

Recently, the usage of plant lecithin as emulsifiers in pharmaceuticals, cosmetics and foodstuffs proved to be of great interest (1–3). In particular, egg yolk lecithin (4) and plant lecithin obtained from soybean are popular (1, 3, 5, 6). The phospholipids structure and their amphiphilic character allow being good surface tension reducing agents and effective emulsifiers (1, 3, 6–8). The native lecithin surface active properties are not always suitable for application in food, pharmaceuticals and cosmetics. The emulsifying properties depend among other properties on the profile of fatty acids in phospholipid structure. Unsaturated fatty acyl groups in phospholipids give the possibilities of chemical modifications such as an increase of the phospholipids surface activity. The alternative rapeseed lecithin, due to lower sensitivity to oxidation (36% polyunsaturated fatty acids) in comparison to soybean (70% polyunsaturated fatty acids), increases likelihood of its usage not only in the technical fields but also in the food, cosmetic and pharmaceutical industries (2, 9, 10).

The chemical modification of plant phospholipids by addition of hydroxyl groups to phospholipids unsaturated fatty acids can reduce the surface tension in modified lecithin aqueous dispersions (1, 11–13). The method of phospholipids hydroxylation is often conducted with 30% hydrogen peroxide in presence of organic acids (Fig. 1) (1, 11, 13–17). The main deficiencies of this method are peroxide by-products and the likelihood of fatty acyl group degradation, which results in low product stability (13, 14). An alternative method is the lecithin hydroxylation based on lecithin chlorination and chlorine replacing with hydroxyl groups in the chlorine derivatives (Fig. 2).

In this study, rapeseed LESF was hydroxylated via two methods. The surface tension reducing properties were compared. The iodine number (IN), peroxide number (PN), acid number (AN), minimal surface tension \( \eta_{\text{min}} \) and the critical micelle concentration (CMC) of LESF and hydroxylated lecithins were determined.

EXPERIMENTAL

Crude commercial rapeseed lecithin free of erucic acid and glucosinolates (00-type rapeseed), purchased from Kama Foods (Brzeg, Poland) was deoiled with acetone and fractionated with 95%
ethanol. LESF was obtained with the method described elsewhere (18). LESF used in experiments was characterized in Table 1. The LESF fatty acids profile (GC) was: 5.6% palmitic, 0.8% stearic, 57.1% oleic, 30.6% linolic and 5.6% linolenic acids and the phospholipids profile (HPLC) was 54% phosphatidylcholine, 3% lysophosphatidylcholine and 13% phosphatidylethanolamine. Hydrogen peroxide 30% and acetic acid 99% of analytical grade were supplied by POCH (Gliwice, Poland). Phospholipids standards were from Sigma (St. Louis MO, USA). All the other reagents and solvents obtained from commercial sources were of analytical grade.

**LESF hydroxylation with hydrogen peroxide**

To 5.0 g of rapeseed LESF dispersed in water (15.0 mL), acetic acid (0.3 mL) and hydrogen peroxide (1.5 g) were added and stirred for 15 min at temperature of 20°C. The reaction mixture was neutralized with 20% sodium hydroxide water solution (1.1 mL) and evaporated under vacuum (40°C, 13.3 hPa). From the dry reaction mixture, phospholipids were extracted three times with 25.0 mL of methanol, the combined extract was filtered, evaporated and dried to constant weight under vacuum at conditions mentioned above. The final product (LESF-OH/H₂O₂) was analyzed.
Hydrogen chloride solution in anhydrous acetic acid in concentration of 83 mg HCl/mL was used as the chlorinating agent. LESF (5.0 g) and acetic acid (30.0 mL) were introduced into a flask fitted with a stirrer and mixed. The chlorinating agent (5.8 mL) was added and the process was carried out at room temperature for 10 h. The reaction mixture was evaporated under vacuum (40°C, 13.3 hPa). Next, the crude product was dissolved in chloroform (40.0 mL) and neutralized with 10% sodium carbonate water solution (20.0 mL). The organic layer was separated, dried with anhydrous calcium chloride (10 g, 1 h) and filtered. The filtrate was evaporated and the residue was dried to constant weight under vacuum at conditions mentioned above. The product (chlorinated LESF) was analyzed.

**Chlorine replacing with hydroxyl group in chlorinated LESF**

Chlorinated LESF (7.0 g) was dissolved in chloroform (20.0 mL) in a flask fitted with a reflux condenser. 1 M sodium hydroxide methanol solution (6.0 mL) was added and the substitution reaction was carried out for 1 h in boiling temperature. The reaction mixture was evaporated under vacuum (40°C, 13.3 hPa) and the solid phase was extracted three times with 20.0 mL of methanol. The combined extract was filtered, evaporated and dried to constant weight under vacuum at conditions mentioned above. The product (chlorinated LESF) was analyzed.

**Iodine, peroxide and acid numbers**

The IN, PN and AN were determined with usage of volumetric pharmacopeial methods (19) using chloroform (IN, PN) and toluene/ethanol 9:1, v/v (AN) as the solvents and 0.01 M, 0.01 M sodium thiosulfate and 0.1 M potassium hydroxide in ethanol for IN, PN and AN, respectively (three measurements for each sample).

**Chlorine content**

Chlorinated phospholipids (1.5 g) and 20% sodium hydroxide (50.0 mL) were introduced into a flask fitted with a reflux condenser and heated at boiling for 1 h. The reaction mixture was quantitatively filtered into volumetric flask and filled with water to 100.0 mL. Chlorine content (three measurements for each sample) was determined by standard Volhard method (20).

**NMR spectroscopy**

^1H-NMR spectra were recorded with a Bruker AM 500 spectrometer at 500 MHz frequency with tetramethylsilane as an internal standard in CDCl₃.

**Surface tension reduction test**

The surface tension reduction of lecithin products aqueous dispersions was determined by stalagmometric method at room temperature using a 5.0 mL volume bulb and tube of 1 mm inner diameter. LESF and its derivatives were homogenized (3000 rpm) and the measurements were obtained after 10 min. The initial dispersion (30.0 g/L) was gradually diluted to 2.0 g/L by every 2.0 g/L. Surface tension of lecithin dispersions was transferred to distilled water (72.6 mN/m) and was plotted against the logarithm of the concentration to determine the η_{min} (mN/m) and CMC (g/L) (4). All measurements were carried out in triplicate for three independent samples of each lecithin product.

**RESULTS AND DISCUSSION**

Hydroxylated LESF obtained by hydroxylation with 30% hydrogen peroxide (LESF-OH/H₂O₂) was a light yellow amorphous semisolid (yield 83%, referred to initial LESF). The low IN = 0.2 g I₂/100 g LESF. The IN, PN and AN of the reaction mixture with a reflux condenser were determined with usage of volumetric pharmacopeial methods (19) using chloroform (IN, PN) and toluene/ethanol 9:1, v/v (AN) as the solvents and 0.01 M, 0.01 M sodium thiosulfate and 0.1 M potassium hydroxide in ethanol for IN, PN and AN, respectively (three measurements for each sample).

**Chlorine content**

Chlorinated phospholipids (1.5 g) and 20% sodium hydroxide (50.0 mL) were introduced into a flask fitted with a reflux condenser and heated at boiling for 1 h. The reaction mixture was quantitatively filtered into volumetric flask and filled with water to 100.0 mL. Chlorine content (three measurements for each sample) was determined by standard Volhard method (20).

**NMR spectroscopy**

^1H-NMR spectra were recorded with a Bruker AM 500 spectrometer at 500 MHz frequency with tetramethylsilane as an internal standard in CDCl₃.

**Surface tension reduction test**

The surface tension reduction of lecithin products aqueous dispersions was determined by stalagmometric method at room temperature using a 5.0 mL volume bulb and tube of 1 mm inner diameter. LESF and its derivatives were homogenized (3000 rpm) and the measurements were obtained after 10 min. The initial dispersion (30.0 g/L) was gradually diluted to 2.0 g/L by every 2.0 g/L. Surface tension of lecithin dispersions was transferred to distilled water (72.6 mN/m) and was plotted against the logarithm of the concentration to determine the η_{min} (mN/m) and CMC (g/L) (4). All measurements were carried out in triplicate for three independent samples of each lecithin product.

**RESULTS AND DISCUSSION**

Hydroxylated LESF obtained by hydroxylation with 30% hydrogen peroxide (LESF-OH/H₂O₂) was a light yellow amorphous semisolid (yield 83%, referred to initial LESF). The low IN = 0.2 g I₂/100 g LESF.
g and high PN = 8.0 mmol O₂/kg of this product suggests that as a result of rapeseed LESF hydroxylation with 30% hydrogen peroxide, all double bonds of unsaturated fatty acids chains in phospholipids molecules were converted to single C–C bonds with hydroxyl groups and by-products with the peroxide groups. The 1H NMR spectral data analysis confirmed the main structure of hydroxylated LESF (LESF-OH/H₂O₂) (δ, ppm): 0.70–2.15 (CH₃ and CH₂ aliphatic protons from saturated acyl groups), 2.15–2.40 (CH₂ aliphatic protons connected with carbonyl groups), 3.50–4.00 (protons from hydroxyl groups), 4.10–4.50 (protons from glycerol CH₂O, CH₂OP) and 5.22–5.41 (CH aliphatic protons connected with OH groups). The total conversion of fatty acids double bonds to single bonds with secondary hydroxyl groups (IN 0.2 g I₂/100 g) has influence on decreasing product stability (possibly due to fatty acid chains degradation) (13). Moreover, lecithin products with the level of peroxides above 5 mmol O₂/kg should not be used in pharmaceuticals, cosmetics and foodstuffs (5). Taking this into account, the above finding for LESF-OH/H₂O₂ range of usage is restricted in many fields (5).

In the alternative method, the intermediate product obtained by LESF chlorination with hydrogen chloride was a solid with IN 50.6 g I₂/100 g and the 9.67% chlorine content. In comparison to initial LESF (IN 68.5 g I₂/100 g) chlorinated LESF has less double bonds in unsaturated fatty acids. In this intermediate product only 26% of double bonds were connected with hydrogen chloride. The final product obtained in reaction of chlorinated LESF with 1 M sodium hydroxide methanol solution, was a yellow amorphous semisolid with IN 61.2 g I₂/100 g and PN 0.2 mmol O₂/kg. It was shown that LESF-OH/Cl was practically free from peroxides and chlorine, and was also free from chloride. The AN of LESF-OH/Cl (16.0 mg KOH/g) is lower than the AN value of LESF-OH/H₂O₂ (25.0 mg KOH/g) and is similar to the value of initial LESF (13.0 mg KOH/g). This results showed that LESF-OH/Cl has less acidic by-products than LESF-OH/H₂O₂, what is significant for application in pharmaceuticals and cosmetics. The increased IN of LESF-OH/Cl (from 50.6 to 61.2 g I₂/100g) may suggest that in the reaction of chlorinated LESF with NaOH methanol solution 21% of double bonds in fatty acids were reconstructed by hydrogen chloride elimination. In comparison to LESF-OH/H₂O₂, the product obtained by chlorine replacing with hydroxyl groups in chlorinated phospholipids (LESF-OH/Cl) contained only 10% of hydroxylated double bonds in fatty acids, what is significant for the product stability (13). The obtained results were also supported by the 1H NMR spectrum (decrease of hydrogen integration number of the signals at 3.66 and 5.20–5.45 ppm compared to NMR spectrum of LESF-OH/H₂O₂). The 1H NMR LESF-OH/Cl analysis showed (δ, ppm): 0.70–2.15 (CH₃ and CH₂ aliphatic protons from saturated acyl groups), 2.20–2.40 (CH₂ aliphatic protons connected with carbonyl groups), 3.50–4.00 (protons from hydroxyl groups), 4.10–4.50 (protons from glycerol CH₂O, CH₂OP) and 5.22–5.41 (CH aliphatic protons connected with OH groups).

CMC is usually utilized as an indicator of the effectiveness of surface tension reduction and helps to identify good emulsifier (8). The surface tension reduction test showed significant differences between rapeseed LESF and the two hydroxylated products (LESF-OH/H₂O₂ and LESF-OH/Cl) (Table 1). Hydroxylated lecithins proved a reduced surface tension at low concentrations. Mean CMC values of the triplicate surface tension tests for LESF-OH/H₂O₂ and LESF-OH/Cl were 6.0 and 9.8 g/L, respectively. Above the CMC value, the surface activity of an emulsifier does not increase with the addition of more emulsifier. Thus, the smaller the CMC of the emulsifier, the better its emulsification ability. Hydroxylated lecithins obtained have much improved surface reduction ability than the rapeseed LESF with CMC 17.8 g/L. The CMC value for initial rapeseed LESF was similar to those reported for soybean lecithin 13.6 g/L (3) and 15.8 g/L (4) as well as for egg-yolk lecithin 15.3 g/L (4). These data may suggest that other nonhydroxylated PC-enriched lecithin fractions also have the worse surface activity in comparison to LESF-OH/H₂O₂ and LESF-OH/Cl. The obtained results also indicate that the surface tensions of the aqueous dispersions of LESF-OH/H₂O₂ and LESF-OH/Cl were reduced to a constant 20.2 and 25.0 mN/m, respectively, when the CMC were reached.

CONCLUSIONS

The hydroxylated derivatives of rapeseed LESF have significantly improved surface tension reduction capability than the initial LESF. Considering the obtained degree of surface tension reduction and the CMC value and the deficiency of peroxides and chlorine, the LESF-OH/Cl should be regarded as more efficient emulsifier in pharmaceuticals and cosmetics than the rapeseed LESF and LESF-OH/H₂O₂.
REFERENCES


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