# ENCAPSULATION OF FISH OIL IN BIODEGRADABLE POLYMERS BY THE PGSS PROCESS

K. Dübbert, N. Rubio- Rodriguez, S. Kareth, S. Beltran, M. Petermann

Fish oil contains high amounts of omega 3 fatty acids, which are used as food ingredients, due to their positive effects and health benefits on human beings. Especially for the prevention of cardiovascular diseases, omega 3 fatty acids are discussed. The use of fish oil in food- and pharmaceutical products is problematic, due to the low oxidation stability of the oil. This leads to a nasty smell or taste of the products. For this reason the improvement of the stability of the omega 3 fatty acids towards oxidation is one of the major tasks in food industry. A high pressure spray technique (PGSS = Particles from gas saturated solution) has been applied to encapsulate fish oil in a biodegradable polymer. As polymer Chitosan, which is a deacetylated derivate of chitin, was used. Chitosan is a Polysaccaride, a natural bioactive macromolecule, which is generally derived from crustacean shell waste. Conventional encapsulation techniques, such as spray drying, need relatively high temperatures, which can lead to oxidation of the oil already during processing. In the PGSS-drying process a supercritical fluid; in this case carbon dioxide, is dissolved in a liquid emulsion which consists of Chitosan, maltodextrin, fish oil, water and an emulsifier. The supercritical gas is contacted with the emulsion in a static mixer. Followed by a rapid depressurization of this emulsion through a nozzel. The water is already extracted by the gas in the static mixer, so that after expansion only a small amount of solvent has to be evaporated in the spray tower. The conditions in the spray tower have to be set in such a way, that condensation is avoided and products with low water residues are obtained and in addition a thermal deterioration of the fish oil is prevented.

The particle size of the obtained particles was measured with a laser diffraction method using a Mastersizer 2000. The morphologies of the particles were analyzed by SEM (scanning electron microscope). The amount of fish oil on the surface of the particles was determined by ultra violet spectrometer (UV). The humidity of the powder was analyzed by evaporating the water in an oven.

The contribution will illustrate the PGSS process, which was used for the encapsulation, and will point out the influence of the main process parameters on particle size, particle morphology, residual humidity and oil content of the fish oil capsules.

## 1. INTRODUCTION

The interest of polyunsaturated fatty acids (PUFA), especially of omega-3 fatty acids, began several years ago and it has led to a wide range of scientific research, as a result of their biological effects and diverse health implications [1]. The omega-3 fatty acids can prevent certain diseases that are common in the western civilization, i.e. they can reduce the risk of heart disease and high blood pressure or protect against cancer [2]. In the last years the intake of the important omega-3 acids has changed. There is a reduced consumption of fish oil which is rich in omega-3 acids, but there is an increasing consumption of vegetable oils which are rich in omega-6 acids. Some diets such as Japanese or Mediterranean recommend a ratio of omega-6 to omega-3 of 5:1 and 10:1 respectively in the diet [3, 4, 5]. The enrichment of foods with omega-3 acids is a recent advancement and now a number of functional foods are being enriched with omega-3 PUFA.

In this work fish oil was selected because of it positive properties for the human body. A formulation that allows protecting the fish oil from high temperatures during the process, oxidation and UV light must be found. Studies have shown that supercritical fluid technology could be an interesting option for omega-3 processing. This process uses low temperatures, which prevent oxidation and minimize the use of organic solvents [6]. Conventional methods in omega-3 processing are extraction of crude fish oil, spray drying and extrusion techniques [7]. The PGSS-drying process uses relatively low temperatures in comparison with other techniques, there is no oxygen in the processing which can influence the oxidative stability of the fish oil, furthermore it is possible to influence the particle size of the produced powder. High pressure technology allows the production of powders with properties difficult to achieve by classical methods.

The production of fish oil as solid particles has an enormous interest in both food and pharmaceutical industries since it allows broadening the application field of marine lipids as active ingredients. This production improves the sensorial properties of omega-3 enriched products and enhances the controlled release in order to optimize the beneficial effects on the human body. A wide range of substances has been proposed in the literature as coating materials for fish oil encapsulation such as proteins, carbohydrates and polyethylene glycols. For this application an experiment was carried out using a multilayer oil-in-water emulsion containing the fish oil (core material), a primary and secondary emulsifier and a mixture of maltodextrine and chitosan (shell material).

The PGSS-drying process (**P**articles from **G**as **S**aturated **P**rocess) is characterized by the use of supercritical solvents. The most common supercritical solvent (Sc-solvent) is carbon dioxide ($CO_2$). $CO_2$ has low toxicity and economical costs, it is not flammable, and it is environmentally friendly. The critical conditions of $CO_2$ ($T_c$=304,15K, $p_c$=7,38MPa) make it suitable for processing thermo-degradable compounds such as polyunsaturated fatty acids.

The produced particles with the PGSS-drying process were analyzed and the results will be discussed in this article.

## 2. MATERIALS AND METHODS

### 2.1 Materials

For this project refined anchovy oil was used (company Denomega Nutritional Oils AS Norway). The selected coating material was maltodextrine (dextrose equivalent (DE) 15-20) provided by Cargill Deutschland GmbH. Chemically this compound represents a high molecular weight polysaccharide. Two components were studied as primary emulsifiers: polysorbate 80 (Tween 80), is a non-ionic surfactant able to produce neutral charged oil drops and lecithin, which is an amphipathic phospholipid that can act as an anionic surfactant producing negative charged oil drops. The second emulsifier was an acid solution of chitosan (2 % wt. in an aqueous solution of 1 M acetic acid).

### 2.2 Preparation of the oil-in-water emulsions

The oil-in-water emulsions were prepared with the layer-by-layer deposition method as previously described in literature [8] (See Figure 1). With this method the emulsion is prepared in three steps that include forming a primary, secondary and tertiary emulsion. This was done by making an initial emulsion consisting of fish oil and emulsifier (Tween 80 or lecithin). The secondary emulsion was formed by diluting the primary emulsion with an acid chitosan solution. The final sample was prepared by mixing in an aqueous maltodextrine solution. In each step the emulsions (2 liter) were mixed mechanically for 5 min at 19000 rpm in an Ultra-Turrax (type: T2 digital) homogenizer. Four different emulsions were studied; the different emulsions are presented in Table 1. The stability of the emulsion was proved at ambient conditions and there has not been any observed phenomenon of creaming for more than one hour.

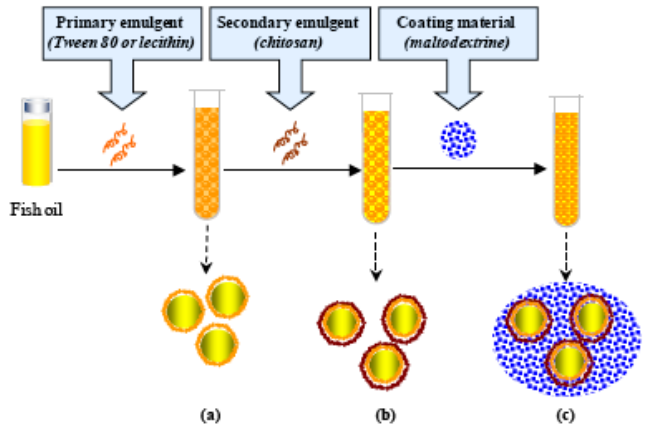


Figure 1: Mechanism of double-layer formation in oil-in-water emulsions. (a) Oil droplets coated by a monolayer of primary emulsifier; (b) Oil droplets coated by a double layer formed from the interactions between primary and secondary emulsifier; (c) Multilayer oil-in-water emulsion in a maltodextrine aqueous solution.

Table 1: Composition of the oil-in-water emulsions prepared for fish oil microencapsulation

| Emulsion | Fish oil (%wt) | Primary emulsifier | | Chitosan (%wt) | Maltodextrine (%wt) | Water (%wt) | Oil/solid ratio | Oil in water (%wt) |
|---|---|---|---|---|---|---|---|---|
| | | Tween 80 (%wt) | Lecithin (%wt) | | | | | |
| A | 5.07 | 0.40 | - | 0.06 | 26.16 | 68.31 | 0.19 | 7.4 |
| B | 8.53 | 0.35 | - | 0.14 | 33.22 | 57.76 | 0.26 | 14.8 |
| C | 2.79 | - | 1.47 | 0.03 | 15.00 | 80.71 | 0.17 | 3.5 |
| D | 3.39 | - | 1.50 | 0.03 | 14.53 | 80.55 | 0.21 | 4.2 |

2.3 Particles from PGSS-drying

This technology was used to process oil-in-water emulsions of fish oil and matodextrine in order to obtain powders. In this spray drying process a supercritical fluid helps to form fine droplets which dry quickly and result in a very fine powder. The liquid substance to be transformed into powder is mixed with the supercritical gas in a static mixer and then expanded through an atomizing nozzle to ambient pressure in a spray tower. Because of the Joule-Thomson effect the gas cools rapidly and particles are formed.

2.4 Particle characterization

Particle size and distribution of the obtained powders were measured by laser diffraction method using the Mastersizer 2000 particle analyzer. The morphology of the particles was detected with pictures of a scanning electron microscope (SEM). The surface moisture content was measured qualitatively with a FT-IR. The amount of oil on the surface of the particles was determined by UV-spectrophotometric method.

## RESULTS

The cladding material used in this case was maltodextrine and the core material was fish oil. Both were used in form of a water/ oil emulsions. To study morphology, particle size distribution, moisture and oil content, different experiment parameters were changed. Different fish oil concentrations, gas to emulsion ratio (GPR), pre-expansion pressures, spray tower temperatures, the static mixing conditions and the primary emulsifier were also changed. The static mixer used in the experiments had a diameter of 8 mm. The results of the experiments are shown in table 2.

The moisture content measured in the obtained samples, was in the range of 2.8 – 7.8 -wt%. With an increasing spray tower temperature the moisture level in the particles is decreasing (see Figure 2). The expansion temperature also has an influence on the moisture. It has been

observed that residual moisture in the powder decreases when expansion temperature increases.

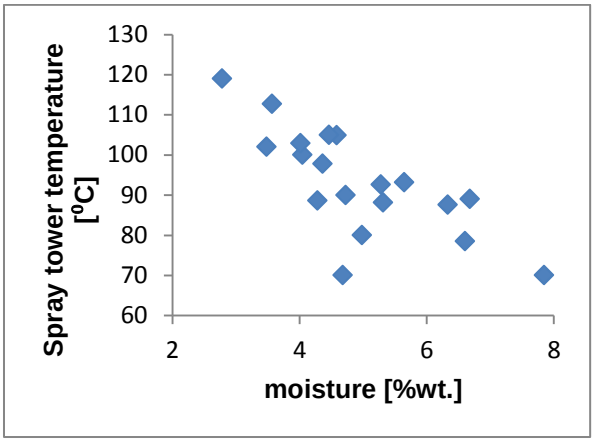


Figure 2: Moisture content (%wt.) as function of the spray tower temperature (°C)

The effect of GPR can be observed in runs 1- 5, 7, 15 and 16. Increasing the GPR value increases the amount of oil on the surface of the particles, presented in Figure 3. The morphology of these particles can be characterized by a spherical structure.

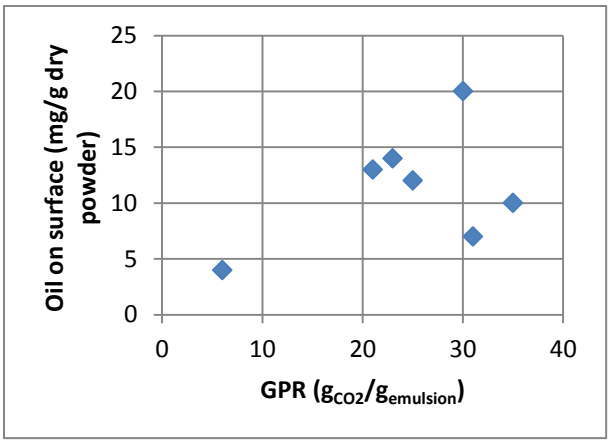


Figure 3: Particle surface oil as function of the GPR ($g_{CO2}/g_{emulsion}$)

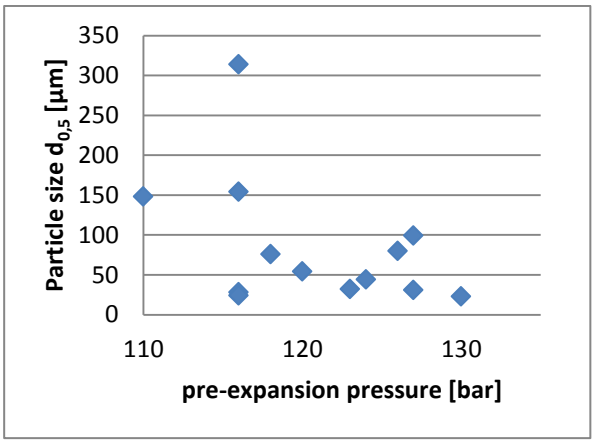


Figure 4: The influence of the pre-expansion pressure on the particle size

There have been observed an influence of the pre-expansion pressure at the particle size (see Figure 4). With a higher pressure the particle size is smaller.

The use of a static mixer in the mixing chamber is another variable process parameter to take into account for the PGSS-drying experiments. Previous studies have reported that working with a static mixer increases the turbulence in the mixing chamber and minimizes the effect of mass transfer resistance, making the contact between $CO_2$ and the aqueous solution more effective [10]. Working with a static mixer promotes the clogging of the mixer chamber and therefore of the nozzle. This was observed in runs 10 – 12. Clogging increases the risk of emulsion destabilization, which explains that even at low pressure and low GPR, the powder

recovered presents a high amount of oil on the surface. The influence of a static mixer can be observed in the morphology at run 10 and 15, see the SEM-pictures in Figure 5. Without a static mixer the particles are spherical, with a static mixer fibers have been observed.

The use of different nozzles has an influence on the particle size, which can be seen from run 8 carried out with the SK-MPF 21 nozzle (slot size 0,89 mm) and run 14 carried out with the SK 27 nozzle (slot size 0,64 mm). In both cases the experimental conditions were similar as well as the solid powder features, but the particle size (comparing the $d_{0,5}$) from run 8 is almost 10 times larger than the particle size from run 14. A similar conclusion can be seen comparing the average particles sizes from runs 4 and 7.

SEM pictures of the particles demonstrate that it is possible to obtain filled micro maltodextrine particles with encapsulated fish oil. The different particle shapes obtained by PGSS-drying are spherical, fibrous, sponge and porous particles. Higher pressure and GPR values promote the formation of a fibrous microstructure (run 21) due to the lower stability of the emulsion, whereas lower pressure and GPR values form spherical microstructures (run 20). An amorphous microstructure with large holes is observed in run 19, carried out with a higher lecithin/ oil ratio (emulsion C).

Holding process variables constant and using lecithin, an ionic emulsifier, rather than Tween 80, a non-ionic emulsifier, it was found that there was a change in curvature of the surfactant layer and destabilization of the emulsion. This may explain why powder obtained in run 20 is made of small agglomerated porous spheres. This leads to poor encapsulation efficiency, or to why the powder obtained in run 21, carried out at higher GPR, is characterized by a fibrous microstructure composed mostly by maltodextrine.

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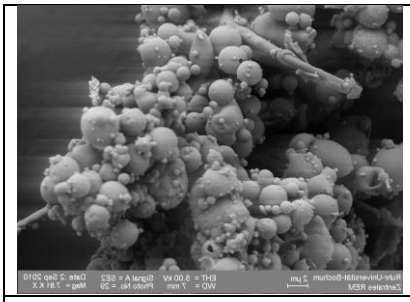
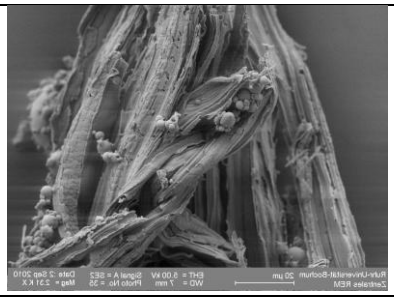
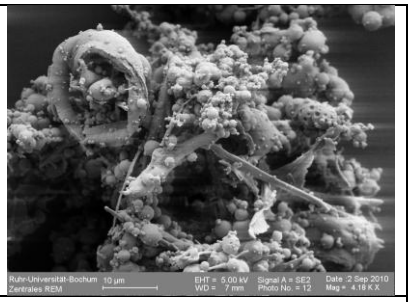
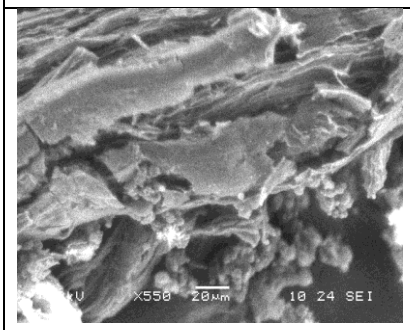
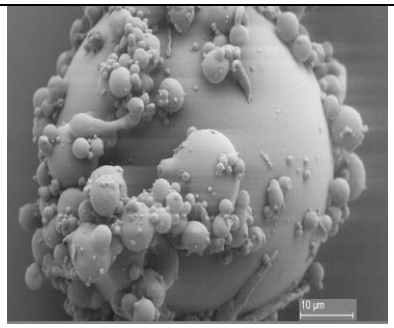
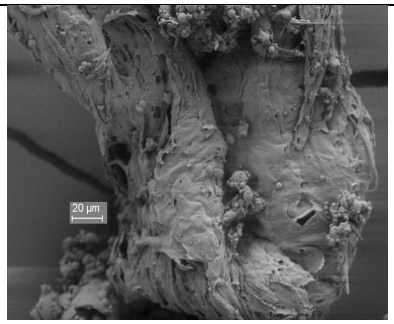
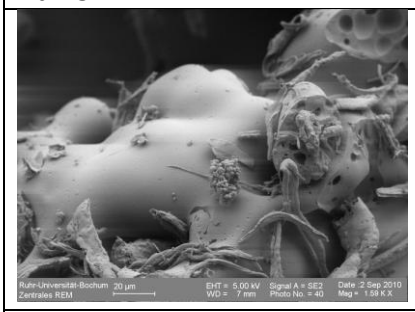
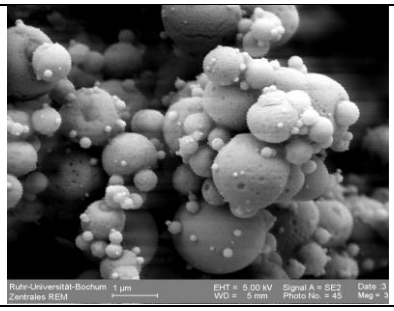
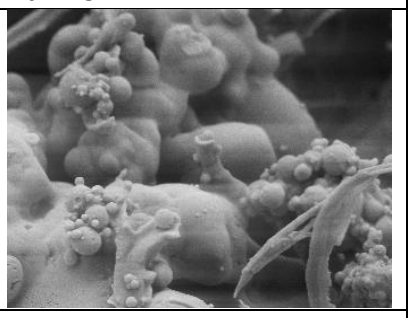
|  |  |  |
| Run 4 | Run7 | Run 8 |
|  |  |  |
| Run 9 | Run10 | Run 15 |
|  |  |  |
| Run 19 | Run 20 | Run 21 |

Figure 5: SEM-Pictures of the produced particles with PGSS-drying

## CONCLUSION

Fish oil encapsulation in a maltodextrine coating has been carried out by PGSS-drying using different multilayer oil-in-water emulsions stabilized with a non-ionic/cationic emulsifier (Tween 80/chitosan) and an anionic/cationic emulsifier (lecithin/chitosan). The encapsulation with fish oil by PGSS-drying is a complex process. The stability of the emulsion is related to the phase behaviour of the $CO_2$/ emulsion mixture, and furthermore from the pre-expansion pressure, pre-expansion temperature and the gas-emulsion ratio (GPR). In experiments with the same temperature, with low pre-expansion pressure and low GPR value, the particles have a spherical structure and the amount of oil on the surface of the particles is low. In this case it was observed that it is better to use a low oil/ water ratio in the emulsion and that the use of a non-ionic biopolymer allows higher emulsion stability in the mixer outlet. With a higher oil/ water ratio, especially at higher pre-pressures and GPR values it was observed that the morphology of the particles is amorphous and porous and the oil encapsulation efficiency was low. Emulsion atomization is controlled by the pressure depletion between the mixer and the expansion chamber and by the nozzle design. At the same conditions, it has been observed that a nozzle with smaller slot diameter allows obtaining smaller particle size. To achieve moisture content less than 3 %wt. the $CO_2$/ water ratio and temperature (363 K) should be high.

Table 2: Results of the PGSS-drying experiments

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Emulsion | A | A | A | A | A | A | A | B | B | B | B | B | B | B | B | B | B | C | D | D |
| Oil/solid mass ratio | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.17 | 0.21 | 0.21 |
| Nozzel | Sk 21 | Sk 21 | Sk 21 | Sk 21 | Sk 21 | Sk 21 | Si | Sk 21 | Sk 21 | Sk 21 | Sk 21 | Sk 21 | Sk 27 | Sk 27 | Sk 27 | Sk 27 | Sk 27 | Sk 21 | Sk 21 | Sk 21 |
| S.m. | no | no | no | no | no | no | no | no | no | yes | yes | yes | no | no | no | no | no | no | no | no |
| P (MPa) | 11.5 | 11.6 | 11.7 | 12.6 | 12.7 | 13.0 | 12.3 | 11.5 | 11.6 | 12.7 | 16.7 | 25.7 | 11.6 | 12.0 | 12.4 | 13.4 | 15.5 | 11.7 | 11.0 | 11.8 |
| GPR (kg $CO_2$/ kg emulsion | 30 | 6 | 21 | 35 | 21 | 88 | 31 | 30 | 50 | 15 | 23 | 18 | 25 | 23 | 13 | 51 | 48 | 29 | 27 | 42 |
| $T_{Spraytower}$ (K) | 88 | 89 | 70 | 100 | 70 | 102 | 98 | 88 | 98 | 79 | 90 | 64 | 105 | 89 | 119 | 103 | 105 | 93 | 80 | 93 |
| Density (kg/$m^3$) | 94 | 276 | 124 | 134 | 260 | 168 | 150 | 134 | 189 | 182 | 150 | n.d. | 162 | 193 | 170 | 189 | 134 | n.d. | 148 | 76 |
| $d_{o,5}$ (µm) | 918 | 154 | n.d. | 80 | 99 | 23 | 32 | 995 | 28 | 31 | 380 | n.d. | 24 | 54 | 44 | 505 | 768 | n.d. | 1085 | n.d. |
| Moisture (%wt.) | 6.6 | 6.7 | 7.8 | 4.0 | 4.7 | 3.5 | 3.6 | 3.6 | 4.4 | 6.6 | 4.7 | n.d. | 4.6 | 4.3 | 2.8 | 3.6 | 4.5 | 5.3 | 5.6 | 5.0 |
| Oil surface (mg / g solid) | 20 | 4 | n.d. | 10 | 13 | 6 | 7 | 8 | 6 | 34 | 3 | n.d. | 5 | 14 | 16 | 11 | 12 | 8 | 9 | n.d. |

S.m.: Static mixer; Nozzel: Spraying systems, SK-MFP 21 = SK 21(0,89 mm) ; Spraying systems SK 21 (0,64 mm); Schlik nozzle (1,2 mm)= Si; n.d.: no data available

## REFERENCES


[1] CHOW, C.K., *Fatty Acids in Foods and their Health Implications*, Marcel Dekker, Inc., New York/Basel, **2000**

[2] CHEN, Y.Q., EDWARDS, I.G., KRIDEL, S.J., THORNBURG, T. and BERQUIN, I.M., *Dietary fat -gene interaction in cancer*, Cancer Metastasis Rev, Volume 26, **2007**, pages 535-551

[3] WHO/FAO, Fats and oils in human nutrition, in: FAO (Ed.), **1994**

[4] AMBRING, A., JOHANSSON, M., AXELSEN, M., GAN, L., STRANDVIK, B., FRIEDBERG, P., *Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3fatty acids, the number of leukocytes and platelets and vascular endothelial growth factor in health subjects*, the American Journal of Clinical Nutrition, Volume 83, **2006,** pages 575-581

[5] FERNANDEZ, P.M.; JUAN, S., *Fatty acid composition of commercial Spanish Fast food and Snack food, Journal of food Composition and Analysis*, Volume 12, Issue 3, **2000**, pages 275-281

[6] ESQUIVEL,M.M.,BANDARRA, N.M., FONTAN, I., BERNARDO-GIL, M. G., BATISTA, I., NUNES, M. L. and EMPIS, J. A., *Supercritical Carbon Dioxide Extraction of SardineSardina pilchardusOil*, Lebensmittel-Wissenschaft und-Technologie, Volume 30, Issue 7, **1997**, pages 715-720,

[7] DRUSCH, S., MANNINO, S., *Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids*, Trends in Food Science and Technology, Volume 20, Issues 6-7, **2007**, pages 237-244

[8] OGAWA, S., DECKER, E.A. and MC CLEMENTS, D.J., *Influence of Environmental Conditions on the Stability of Oil in Water Emulsions Containing Droplets Stabilized by Lecithin-Chitosan Membranes*, Journal of Agricultural and Food Chemistry, Volume 51, **2003**, pages 5522-5527

[9] Weidner, E., *High pressure micronization for food applications*, The Journal of Supercritical Fluids, Volume 47, Issue 3, **2009**, pages 556-565

[10] MARTIN, A., PHAM, H.M., KILZER, A., KARETH, S. and WEIDNER, E. *Micronization of polyethylene glycol by PGSS (Particles from Gas Saturated Solutions)-drying of aqueous solutions*, Chemical Engineering and Processing: Process Intensification, Volume 49, Issue 12, **2010**, pages 1259-1266