EFFECT OF POLYMER ON THE MICROENCAPSULATION OF FISH OIL BY SPRAY DRYING AND SUPERCRITICAL ANTI-SOLVENT PROCESSES AND CHARACTERIZATION OF FISH OIL POWDER

BY

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ABSTRACT

The demand for small particle engineering together with biocompatible or biodegradable carrier material to produce micro and nanoparticles is widely employed in the pharmaceutical, cosmetic and food industries. In this study, supercritical fluid precipitation and particle formation of menhaden fish oil using the supercritical anti-solvent method was developed which was compared with that of micro-encapsulation of the fish oil by spray drying method which is a continuing process to produce products with functional properties. Menhaden Fish oil (20 - 30% Omega-3) was used and encapsulated with two carrier material hydroxy propyl methyl cellulose (HPMC) 15 cP and HPMC 5 cP as a solid carrier and PEG 6000 as a plasticizer. Moreover, the effect of polymer composition on physicochemical characteristics of fish oil microcapsules produced by spray drying and supercritical anti-solvent (SAS) process was also investigated. The solid content (wt./vol. %) was in a range from 6.5 to 10.25% for all the formulations. Response surface methodology (RSM) was employed to optimize the encapsulation process of fish oil by spray drying technique where inlet air temperature and feed emulsion rate were used as the variables. The encapsulation efficiency (EE) of spray dried fish oil powder had an optimum value of 75% which indicated a promising feature of the microencapsulation process. Based on the optimum condition (inlet air temperature of 186 ^{o}C and emulsion feed rate of 404.4 mL/hr), eight (8) formulations (AF1-SD – AF4-SD, BF1-SD – BF4-SD) were selected. The same eight formulations were also produced by SAS process and it was found that the ratio and concentration of the polymer to the lipid phase influenced the reconstitution properties of fish oil powder. Scanning electron microscopy, EE and peroxide value conducted in this study revealed that the encapsulated oil produced by SAS process provided the highest protective and prolonged effect on the masking of fish oil aroma. Moreover, all the indices of powders prepared from HPMC 15 cP and HPMC 5 cP showed that the stability of the microencapsulated fish oil increased which was determined at 7d intervals over a 28d period. Microencapsulation produced by SAS process with high solid content (10.25 wt./vol. %) provided a stable fish oil powder compared with less solid content formulations. Among the formulations, AF1-SAS containing high concentration of HPMC provided highest encapsulation efficiency of 82% with very low peroxide value (5 mEq O_2/kg oil) over a 28d period. It can be concluded that SAS process gave more stable powder particles than spray drying and can be recommended as a method to produce particles with long-term stability.

خلاصة البحث

شهدت الحاجة لهندسة الجزيئات الدقيقة المتوافقة بيولوجياً أو القابلة للتحلل الطبيعي لإنتاج جسيمات ميكرو أو نانو تطبيقات واسعة في المجالات الصيدلانية، صناعة التجميل، والصناعات الغذائية. في هذه الدراسة، تم ترسيب السوائل فوق الحرجة وتكوين جسيمات من زيت سمكة مينهادن باستعمال طريقة ضد–المذيب فوق الحرج وتمت مقارنتها مع طريقة تغليف المايكروكابسول لزيت السمك بواسطة الرش الجاف والتي بدورها تشكل طريقة لإنتاج منتجات ذات خصائص فنية. زيت سمكة المينهادن (**20–30%** أوميجا–**3**) استعمل لتكوين كبسولات مع حاملين آخرين هما بروبيل ميثيل سيليلوز **15**cP (HPMC) و HPMC **15**cP كحتمل صلب و PEG6000 كملدن. تمت دراسة تأثير مكون البلمر على الخصائص الفزيوكيميائية للمايكروكبسولات المنتجة بطريقة الرش الجاف و طريقة ضد–المذيب(SAS) أيضاً. مدى المحتوى الصلب (wt.%) كان من **6.5** إلى **10.25%** لجميع التحضيرات. تم توظبف طريقة إستجابة السطح (RSM) لتحسين عملة كبسلة زيت السمك بواسطة الرش الجاف، حيث استعملت الحرارة الداخلية ومعدل إطعام المستحلب كمتغيرات. كفاءة الكبسلة لبودرة زيت السمك المعدة بالرش الجاف تحصلت على كفاءة **75%** مما يجعلها خاصية واعدة لطريقة الكبسلة المايكروية. باعتماد الظروف المعيارية (الحرارة الداخلية **186** س° ومعدل تغذية المستحلب **404.4** مل/ساعة)، تم اختيار **8** تحضيرات (AF1-SD – AF4-SD, BF1-SD – BF4-SD). ونفس هذه التحضيرات تمت إعادة إعدادها بوساطة طريقة SAS ووجد أن معدل و تركيز البلمر بالنسبة للجزء الدهني أثّر على خصائص إعادة إحلال بودرة زيت السمك. مسح المجهر الإلكتروني، EE و قيمة البيروكسايد المتحصل عليها في هذا البحث أثبتت أن الزيت المكبسل والمنتج بطريقة SAS يعطي أعلى قيمة حماية و أطول تأثير لتغطية رائحة زيت السمك. بالإضافة ألى أن كل المؤشرات للبودرة المنتجة من HPMC 15 cP و HPMC 5 cP أظهرت أن ثباتية زيت السمك المعد بطريق المايكروكبسول ازدادت، وهذا تحقق عند فترة **7**أيام خلال فترة **28** يوم. المايكروكبسول المنتجة بطريقة SAS مع محتوى صلب مرتفع (10.25 wt./vol. %) أعطت بودرة زيت سمك ثابتة إذا ما قورنت مع تحضيرات أخرى بمحتوى صلب أقل. من كل التحضيرات، أعطت AF1-SAS المحتوية على تركيز عالي من HPMC أعلى نسبة فعالية لعملية الكبسلة بنسبة **82%** مع قيمة منخفضة جداً للبيروكسايد (5 mEq O_2/kg oil) لفترة **28** يوم. خلصت هذه الدراسة إلى أن طريقة SAS أعطت جزيئات بودرة أكثر ثباتاً مقارنةً مع طريقة الرش الجاف، وبالإمكان التوصية بها كطريقة لإنتاج جزيئات ذات ثباتية طويلة الأمد.

APPROVAL PAGE

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Fish oil is considered to be a good source of omega-3 and 6 polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that have been shown to reduce the risk of many diseases, and to improve the central nervous system (Stone, 1997; Salem et al., 1998; Simopoulos et al., 1999). The most well- known and widely researched omega-3 PUFAs are eicosapentaenoic acid (EPA, 5 double bonds) and docosahexaenoic acid (DHA, 6 double bonds).

Despite continuous research revealing numerous health benefits associated with consumption of omega-3 PUFAs, most diets contain below the recommended amount (Kris-Etherton et al., 2000; Jin et al., 2007). Embellishment of foods with fish oil is one of the ways to enhance the level of omega-3 PUFAs in the diet (Fereidoon et al., 1998; Wallace et al., 2000). Embellished foods should not be stored for long periods or exposed to high temperatures that could speed up deterioration (Lovegrove et al., 1997; Kolanowski et al., 1999; Keogh et al., 2001). In some research works, it was found that the dietary intake of omega-3 PUFAs is equally effective in encapsulated fish oil and enriched foods (Wallace et al., 2000; Krawczyk, 2001; Liu et al., 2001). The main drawback of food embellishment with omega-3 PUFAs is the unpleasant flavor of fish oil, which has a negative impact on the product acceptability to the consumer (Krawczyk, 2001). The unpleasant off-flavor can be avoided by encapsulation with polymers and the fish oil can be stabilized by the addition of antioxidants (Neil and Younger, 1998).

The most significant challenge for processing omega-3 is preventing its degradation because the high content of unsaturated double bonds that are highly susceptible to oxidative degradation and they are also thermally labile. Autoxidation is known as the most common process leading to oxidative deterioration. Fish oils, which have high PUFA content, have the potential for being broken down into smaller molecule by this process. Also, attempting to supplement foods with PUFA may lead to only limited success due to both their low solubility in most food systems and excessive susceptibility to oxidation (Aghbashlo et al. 2012). Oxidation of omega PUFA sources can be prevented through the use of controlled storage conditions (eg. packing in an inert atmosphere and chilling), through the addition of antioxidants, and by microencapsulation.

Controlled storage can be expensive and time consuming if the atmosphere must be repeatedly modified upon opening and closing of ingredient storage containers. Selecting an effective antioxidant or blend of antioxidants is challenging to control the stability of bulk oils. Throughout the stages of product development and storage, it is highly possible that the omega-3 PUFA source might see many of these different conditions of oxidation. Furthermore, several commonly used antioxidants (tocopherols for example) can act as pro-oxidants at high levels. When the omega-3 PUFA source ingredient is combined with other ingredients also containing antioxidants, it is possible that antioxidant content may increase to a level where pro-oxidant effects occur.

Microencapsulation refers to surrounding or embedding the oil in a matrix typically composed of proteins or carbohydrates and can be accomplished through a variety of processing techniques. In theory, microencapsulation protects the core material against degradation by light, heat, and oxygen; however, microencapsulation does not always produce a product that is more stable than the non-encapsulated form. Those food ingredients that may benefit from encapsulation include flavors, acids, alkalis, buffers, lipids, enzymes, microorganisms, artificial sweeteners, vitamins, minerals, preservatives, antioxidants, cross-linking agents, leavening agents, colorants, and nutrients (Barbosa-Canovas, 2005). Stability of encapsulated lipids depends on properties including oil distribution within the particle, particle size and surface area, particle density, wall material composition (glass transition temperatures, crystallinity, extent of interaction with the core material), moisture content, and water activity. If processing conditions and wall materials are selected appropriately, microencapsules with long term stability can be prepared. Microencapsulation can also facilitate incorporation of oily ingredients into food matrices as it transforms the lipid into a dried powder. Encapsulation might also mask undesirable flavors and odors associated with omega-3 PUFA sources (Wakil et al., 2010; Sanguansri and Augustin, 2006).

Microencapsulation has become an active field of research and innovation during the last few decades to convert liquid food ingredients like essential oils and flavorings into dry powder particles (Zhongxiang and Bhandari, 2010; Gharsallaoui et al., 2007). The purpose of microencapsulation is to protect the fish oil against oxidation, thus increasing the shelf life while stored in a cool and dark place under vacuum (Young et al., 1993; Rabiskova et al., 1994; Heinzelmann et al., 2000).

Among all the microencapsulation techniques, spray drying is the most common and recognized technology used in food as well as in pharmaceutical industry due to its efficiency and capability to produce good quality powder at minimal cost (Ashady et al., 1993). Another recognized technology used in the pharmaceutical industry as well as in the food industry is supercritical anti-solvent (SAS) process. Supercritical anti-solvent processes are based on solution of the solutes into the conventional liquid solvent using supercritical fluid. The supercritical fluid saturates the liquid solvent resulting in the precipitation of solute by an anti-solvent effect.

Fish oil can be encapsulated with different types of wall materials such as hydroxypropyl methyl cellulose, methyl cellulose, maltodextrin, derivatized starches, pectin, gum arabic, alginate, whey protein isolate, corn syrup solids etc (Risch and Reineccius, 1995; Ré, 1998; Madene et al., 2006; Gharsallaoui et al., 2007; Jin et al., 2008). An ideal wall material would be one that forms a fine and stable emulsion, forms microcapsules with high encapsulation efficiency (low surface oil content) at high oil:wall ratios; produces a glassy shell capable of preventing oxidative degradation of the encapsulated material, and maintains structural integrity throughout long term storage. In general, the following characteristics are desirable for encapsulation of omega-3 PUFAs by spray drying: emulsifying capabilities, good film forming abilities, water solubility, bland flavor/sensory acceptability, barrier properties (water vapor and oxygen), low cost, and compatibility with regulatory and labeling requirements.

Hydroxypropyl methyl cellulose (HPMC) is a water soluble cellulose ether with a molecular weight of 86000. It is a partly O-methylated and O-(2-hydroxypropylated) cellulose which is available in several grades that vary in viscosity and degree of substitution. Because of the different content of hydroxypropyl and methoxyl in the structure of HPMC, various grades of products of HPMC are available. Based on the availability of different grades, different countries have different pharmacopoeial specifications for models and expression of HPMC. PhEur 2002 describes HPMC by indicating the apparent viscosity in mPa.s (e.g. 2% w/w aqueous solution at 20 ^{o}C) and USP 32 defines HPMC by appending a four-digit number with the name (e.g. hypromellose 2910). Qin et al. (2004) reported the reasons behind the worldwide acceptance of HPMC as a carrier material as:

1. It does not interfere with tablet disintegration and bioavailability characteristics.
2. It has flexibility and solubility in gastro intestinal fluid.
3. It is non-toxic and non-irritating as it is not considered to be a hazard for health.
4. It is tasteless and odorless.
5. It is very stable even though when exposed to light, heat or air including long term storage.
6. It is neither absorbed nor metabolized in the body, so it can be considered as a safe medicinal preparation material.

The primary research goal of this work is to evaluate HPMC as a carrier material for the encapsulation of fish oil using spray drying and supercritical anti solvent processes.

1.2 STATEMENT OF THE PROBLEM

Fish oils are considered to be functional foods as they are a rich source of omega 3, 6 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Erkkila et al., 2006). The extreme sensitivity of fish oils to oxidation can easily lead to the development of off-flavors and cause significant loss of product quality, stability, nutritional value, and bio-availability and the overall acceptability of the food products (Nawar, 1996; Watkins & German, 1998).

Consequently,microencapsulation has been studied to encapsulate omega-3, 6 (ω3, 6) fatty acids to prevent oxidation and to improve stability and bioavailability (Wakil et al., 2010; Sanguansri and Augustin, 2006). Microencapsulation can also facilitate incorporation of oily ingredients into a variety of food matrices as it transforms the lipid into a dried powder. Encapsulation may also mask undesirable flavors and odors associated with ω3, 6 PUFAs sources. As the high price of soft gel capsule of fish oil and the sources of raw materials (animal skin, bones, tendon) of the gel with the formulations of the shell of gelatin capsule becomes questionable for halal tayyiban and the maintenance of soft gel capsule machine is very expensive.

So, the aim of this study is to covert the fish oil into powder form i.e. encapsulation of fish oil with carrier materials using newly developed method of supercritical anti-solvent process. Moreover, the spray drying process is also used to improve the encapsulation of fish oil using different carrier materials. The comparison between the supercritical anti-solvent and spray drying process is also studied in terms of the characterization of powder particles. So, the supercritical anti-solvent process is hopefully providing a new alternative in encapsulation of omega-3 before it can be used into any new food system.

1.3 RESEARCH OBJECTIVES

The study has the following objectives:

1. To study and optimize the method of encapsulation of fish oil with Hydroxy Propyl Methyl Cellulose (HPMC) 15 cP and HPMC 5 cP using spray drying process and to examine the influence of different ratios of wall materials on the properties of encapsulated fish oil.
2. To develop a process of encapsulation of fish oil with HPMC 15cP by supercritical anti-solvent process using carbon dioxide as a supercritical fluid and to determine the effect of different ratios of polymers on the encapsulation of fish oil.
3. To develop a process of encapsulation of fish oil with HPMC 5cP by supercritical anti-solvent process using carbon dioxide as a supercritical fluid and to determine the influence of composition of polymers on the encapsulation of fish oil.
4. To compare and contrast the two processes of supercritical anti-solvent and spray drying in terms of the characterization parameters and product properties.

1.4 RESEARCH QUESTIONS/ RESEARCH HYPOTHESIS

Long chain polyunsaturated omega-3 fatty acids were first recognized for their health beneficial effects in the 1970's, when Bang and Dyerberg (1972) studied plasma lipids and lipoproteins linked to the development of cardiovascular diseases in populations with very different intakes of marine omega-3 fatty acids. Since then, fatty acids are thought to provide a wide range of health benefits, including a lower risk of coronary heart disease and improvement in cholesterol. (Perica et al., 2011). The interest and the market for omega-3 enriched foods have therefore developed rapidly during the last decade. One reasonable approach is the addition of a fish oil-in-water (o/w) emulsion, a so called delivery emulsion, as opposed to adding neat fish oil to the food product. In a delivery emulsion, a membrane is created around the oil droplet, which may shield the lipids from its surroundings. Thus, to understand these observations and to improve delivery emulsions for future use, more knowledge is needed and the simple o/w emulsions must be utilized to limit the complexity of influencing factors and thereby increase the possibility of scrutinizing the oxidation mechanisms in more detail. The PhD work set out to test the following hypothesis:

1. As alternative to conventional processes, this study proposes the use of non-conventional method based on supercritical fluid technology in order to obtain the encapsulated form of fish oil. The present research work is focus on the study of novel production process of encapsulation of fish oil through a green technology, supercritical anti-solvent (SAS) process.
2. The type and composition of carrier material, hydroxypropyl methyl cellulose (HPMC) affects the encapsulated fish oil and thereby physical and chemical properties. They will thereby differently be affected in terms of different characterization parameters.

1.5 SIGNIFICANCE AND EXPECTED OUTCOMES OF THIS STUDY

1. The use of omega-3 concentrates as ingredients in food products usually requires a formulation as a dry powder in order to enhance the dispersion of the lipid extract within the food matrix and mask the sensorial impact of the fishy odor compound. Thus, the aim of this study is to use this knowledge for preparing on how the choice of emulsifier, homogenization equipment and emulsification conditions influence stability of the simple emulsion systems.
2. This study also demonstrates that the supercritical anti-solvent process is a promising alternative method for the encapsulation of fish oil with hydroxypropyl methyl cellulose (HPMC) and could lead to application in the food industry improving the stability of fish oil and other essential oils.
3. It is expected to obtain food grade green micronized particles of fish oil from both spray drying and supercritical anti-solvent technique which are relatively inexpensive and organic residues-free that could be regarded as green products.
4. The most effective techniques between the spray drying and supercritical anti-solvent could be recommended for application in the pharmaceutical and nutraceutical industries.