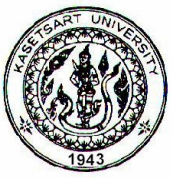
THESIS

DEVELOPMENT OF AN EXTRUDED SNACK SUPPLEMENTED WITH FISH PROTEIN AND N-3 FATTY ACIDS

NANTIPA PANSAWAT

GRADUATE SCHOOL, KASETSART UNIVERSITY

2007



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THESIS

DEVELOPMENT OF AN EXTRUDED SNACK SUPPLEMENTED WITH FISH PROTEIN AND N-3 FATTY ACIDS

NANTIPA PANSAWAT

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Agro-Industrial Product Development)
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Nantipa Pansawat 2007: Development of an Extruded Snack Supplemented with Fish Protein and n-3 Fatty Acids. Doctor of Philosophy (Agro-Industrial Product Development), Major Field: Agro-Industrial Product Development, Department of Product Development. Thesis Advisor: Associate Professor Anuvat Jangchud, Ph.D. 160 pages.

This research aimed to develop a nutritious snack and investigated the effects of extrusion on n-3 fatty acids. The survey showed the consumers were aware of n-3 fatty acids and willing to buy a snack containing n-3 fatty acid from fish oil. A formulation containing rice flour, fish powder, menhaden oil and vitamin E was extruded using a co-rotating twin-screw extruder. Extrusion variables were barrel temperature (125-145°C), screw speed (150-300 rpm) and feed moisture (19-23 g/100g db). Response surface methodology (RSM) was used to study the effects of extrusion conditions on eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and vitamin E contents, physical and sensory properties of extrudates. After extrusion, EPA+DHA contents reduced from 925 mg/100g to 702-948 mg/100g (80-102% retentions). EPA and DHA contents after extrusion were 278-358 mg/100g (71-94% retentions) and 433-591 mg/100g (80-108 % retention), respectively. The contour plots generated from second order polynomial models of EPA+DHA contents suggested that increased screw speed at low feed moisture and increased feed moisture at low screw speed increased EPA+DHA retention. The vitamin E contents (total tocopherols and tocotrienols) in the extrudates were 3.32-2.36 mg/100g (76.9-54.7% retentions). Higher retentions of vitamin E were found at high screw speed and high feed moisture. The contour plots revealed barrel temperature (125-145°C) had minimal affects on the EPA, DHA, and vitamin E retentions as well as the physical properties. The extrudates with low product density, high expansion ratio and low shear strength were found at high screw speed and low feed moisture. Higher degree of likeness in overall sensory characteristics of the extrudates rated by Asian untrained panelists was found at higher screw speed and lower feed moistures. The optimum extrusion conditions obtained by considering the EPA+DHA retentions and the overall liking scores were at the screw speed of 240-300 rpm and moisture content of 19.0-19.5 g/100g (db). The verification runs at conditions in the optimum region were successfully performed.

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October, 2007

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INTRODUCTION

Extrusion is a powerful food processing operation which utilizes high temperature and high shear force to produce a product with unique physical and chemical characteristics. Extruded products are mainly produced from cereal grain and occasionally supplemented with vegetable protein. However, cereal-based snacks are usually low in nutritive density, especially in protein content and essential amino acids (Jean *et al*., 1996). Snack products, which contain mainly carbohydrate and fat, can be made with increased protein content by adding high quality protein including legume (peanut, soybean and cowpea) as well as fish, pork, beef and chicken. Such products must retain satisfactory sensory acceptability (Suknark *et al*., 1999).

Fishery industries, worldwide, generate by-catch and processing wastes that are generally not further processed into value added products. Such raw material can be processed into fish protein concentrate with excellent sensory properties. In this respect, fish protein concentrate represents an ideal processed, protein source to increase the protein nutritive properties of extruded products (Venugopal and Shahidi, 1998). Further, fish protein concentrates that are not completely defatted can add a fish–like flavor to products which is desirable in many areas of the world.

n-3 Fatty acids, namely, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been the subject of considerable research over the past several decades. Their potential to prevent cardiovascular disease and beneficial effects on brain, optic nerves and immune response systems have gained the interest of food processors, the medical community and consumers. Many studies and clinical investigations have been carried out on the metabolism of polyunsaturated fatty acids (PUFA) in general and n-3 fatty acids in particular. n-3 Fatty acids are essential for normal growth and development and may prevent or moderate coronary artery

disease, hypertension, diabetes, arthritis, others inflammatory and autoimmune disorders and cancer (Simopoulos, 2000).

The predominant sources of n-3 fatty acids in human diets are vegetable oils and fish. However, fish is the major source of EPA and DHA (Kris-Etherton *et al*., 2000; 2002). Therefore, raw materials from fish are not only excellent sources of high nutritional value protein but also provide excellent sources of n-3 fatty acids. Unfortunately, n-3 fatty acids are sensitive to oxidation because of their polyunsaturation. The oxidation of fatty acids produces free radicals and rancid flavor that decrease consumer acceptance and lead to rapid deterioration of nutritional quality (Camire, 2000).

Rice is a staple food for Thai people and also a major agricultural product of Thailand. Fewer rice based extruded snacks are currently available compared to extruded corn and wheat products (Tuley, 1992). Product characteristics from rice and other starchy ingredients depend on physicochemical changes that occur during extrusion. The dependent process variables such as barrel temperature, screw speed, screw configuration and die geometry affect system parameters such as mechanical and thermal energy input and residence time (Choudhury and Gautam, 1998). However, due to the ability to incorporate such variables into an optimized extrusion process, products with desirable characteristics are usually obtainable. There are the possibilities of producing puffed snacks from rice and fish protein blends by using both single-screw and twin-screw extruders. However, the twin-screw extruder allows greater flexibility (Gogoi *et al*., 1996a). There are some studies using rice flour and fish muscle (Gogoi *et al*., 1996b) or fish powder (Charoenphol *et al*., 1992) to produce high protein extruded snacks with consumer acceptability. Suknark *et al*. (1998, 1999, 2001) used twin-screw extrusion to produce snack-like products using minced channel catfish (*Ictalurus punctatus*) muscle in combination with partially defatted peanut flour and tapioca starch. These snacks were acceptable to both American and Asian consumers and adaptable to fortification with retinyl palmitate and mixed tocopherols.

From this point of view, there is a potential for developing a new product using low cost or unused fishery raw material as a protein source, rice and n-3 fatty acids from the fish oil as the primary ingredients. n-3 Fatty acid rich fish oil can be added to develop a product with significant amounts of n-3 fatty acids on a per serving basis. The optimum formulas and processing conditions need to be defined. Furthermore, during extrusion, the feed material is subjected to a combination of high shear, temperature and pressure which can affect the nutritive value. Especially, the changes in fatty acid profiles of the extruded product need to be studied.

OBJECTIVES

1. To study the attitude of Thai consumers on rice flour based snacks containing fish and fish oil.

2. To develop an expanded snack supplemented with fish Protein concentrate and n-3 fatty acids.

3. To determine the effects of extrusion on added n-3 fatty acids and vitamin E added as an antioxidant.

LITERATURE REVIEWS

Extrusion

1. Definition

Extrusion is generally described as a thermal mechanical processing operation in which raw material is fed into a hopper and forced by a rotating screw through a heated stationary barrel. The thermally processed material is forced through the die of a specific shape and usually expands or puffs due to the pressure differential as the product exits the die (Kokini *et al*., 1992). Therefore, food extrusion can be defined as a process in which food material is forced to flow, under a variety of conditions including mixing, heating and shear through a die which is designed to form or give shape to the product and/or to puff or expand the cooked product (Rossen and Miller, 1973; Riaz, 2000).

2. Extrusion Products

Extrusion cooking has been used to produce a wide variety of food products and specialty ingredients (Guha *et al*., 2003). The process is versatile, usually economical, with the ability to convert inexpensive raw materials into value added products (Falcone and Philips, 1988; Yeh and Jaw, 1999). Specific process effects include agglomeration, degassing, dehydration, expansion (puffing), gelatinization, nutritional improvement, texturization and cooking (Riaz, 2000). During extrusion, food materials are subjected to high temperature and pressure in combination with shearing stress. Physical and chemical changes include starch gelatinization, protein denaturization and many other macro- and micronutrient interactions. Such changes introduce desirable appearance, aroma, flavor and texture as well as improvement of nutritional properties such as protein digestibility (Chen *et al*., 1991).

The common uses of extruders in the food industry include production of textured vegetable protein (TVP), ready-to-eat (RTE) breakfast cereal, many direct

expanded (DX) and third generation (3G) snacks. First generation snacks are conventional potato chips and baked crackers, second generation are direct expanded snacks and third generation refers to semi-products or pellets, half-products or intermediate-products which are dried to stable moisture content to increase shelf-life. These products are expanded by using hot oil, hot air puffing or microwaving. Extruders are also used to process baby foods, various types of grain/ legume analogs, stabilized cereal bran, produce precooked or thermal modified starches, flours and grain, beer powders, beverages, cheese and casein, food gums, reformed fruit bits and sheets, topping and bakery analogs, coextruded products, precooked pasta, breading and bread-like product (Sevatson and Huber, 2000).

3. Types of Extruders

The history of extrusion was detailed by Riaz (2000). The first hand operated extruder was of piston design and used in 1797 to produce lead pipe. The first food application of extrusion was for pasta production. In the 1930s, the single-screw continuous pasta press was developed. Expanded corn meal snacks were produced in the 1940s by single screw extrusion. Twin-screw extruders were developed later and are preferred for most food applications because of their process versatility (Sevatson and Huber, 2000).

Extruders can be classified into the following categories:

a. Hydraulic ram extruders in which a piston forces the dough to pass through a die (Frame, 1994).

b. Roller extruders that consist of single or twin screws that rotate in the barrel to force the dough through the die. Such extruders can be sub-categorized based on screw design such as segmented vs. solid screw and wet vs. dry extrusion ability.

c. Shear generation characteristics of the design provide for additional categorization. A wide range of shear generation design allows for the following process abilities: cold forming, high pressure forming, low shear cooking, collet extrusion and high shear cooking. Low shear extrusion prevents cooking of the dough; whereas, high shear extrusion provides sufficient mechanical energy for heat generation to cook the dough.

d. The mechanism of heat generation can be used to categorize extruders into adiabatic, isothermal and polytrophic classes. Adiabatic or autogeneous extruders develop the necessary heat required for the process by the friction between the extrudate and the barrel. Isothermal extruders operate at a uniform product temperature throughout the entire length of the barrel. Isothermal extruders are mainly used for forming. Polytrophic extruders have an external heat source and cooling system that provide heat or cooling as required by the specific process.

3.1 Single-Screw Extruders

A single screw extrusion is designed with one screw to move the dough through the barrel (Figure 1). The friction of the material against the barrel wall keeps the material from turning with the screw. The typical single-screw extruder consists of three zones (feeding zone, kneading zone and cooking zone) to accomplish the process objectives (Frame, 1994). In the feeding zone, preconditioned materials are transported into the interior chamber through the feeder. Water is typically injected in the feeding zone of the barrel to alter textural and viscosity development and to enhance the heat conductivity. Compression produced by the feed volume and screw movement increases the shear in the barrel. The extrudate begins to lose granular characteristics, increases in density and pressure develops in the barrel. When the extrudate moves through the kneading zone, it will reach maximum compaction. The shear is usually moderate and the extrudate temperature continues to increase. In the cooking zone, temperature and pressure rapidly increase. Shear rates are highest in this zone. Amorphous changes and/ or texturization occurs.

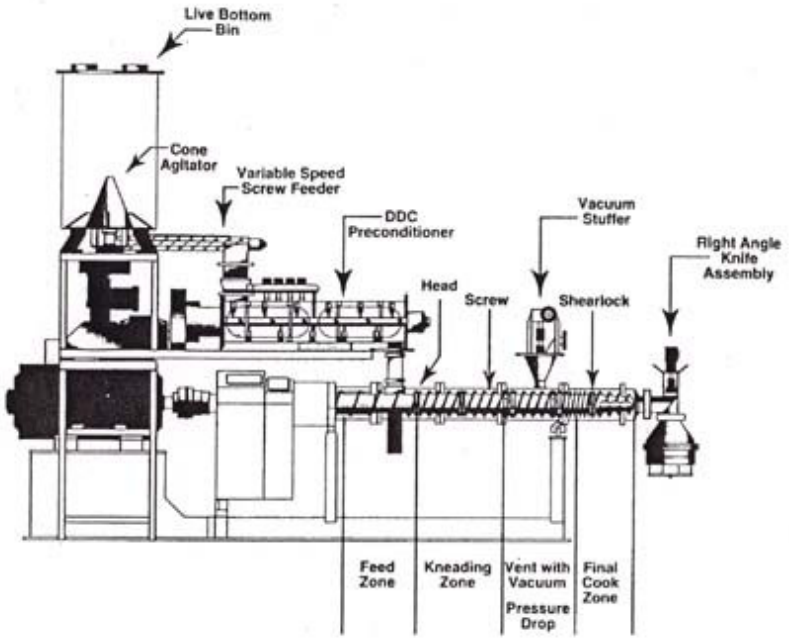


Figure 1 Single screw extruder

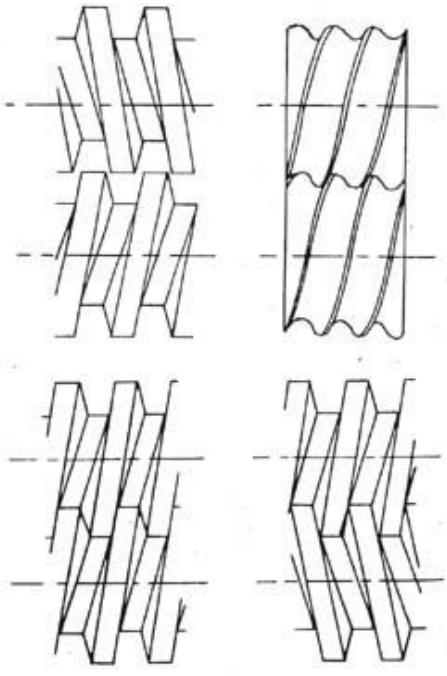


Figure 2 Twin-screw extruder

The temperature and the pressure create the desired final product texture, density, color and functional properties as expansion occurs at the die exit.

3.2 Twin-Screw Extruders

Twin-screw extruders (Figure 2) are classified as counter-rotating twin-screw or co-rotating twin-screw extruders. In the counter-rotating twin-screw extruder, screws turn in opposite directions to force the materials forward. In contrast, both of the screws turn in the same direction to force the materials forward in the co-rotating twin-screw extruder. Counter-rotating twin-screw extruders are good in processing non-viscous materials requiring low speed and long residence time such as the production of gum, fruit jelly strands and licorice confections. Co-rotating twin-screw extruders are widely used in the food industry because the system provides pumping efficiency, good control of residence time distribution, self-cleaning and uniformity of processing. Co-rotating twin-screw extruders can be operated at higher screw speeds than counter-rotating twin-screw extruders because radial forces are more uniformly distributed (Frame, 1994; Riaz, 2000).

Currently, twin-screw extruder technologies are rapidly developing. Twin-screw extrusion allows great flexibility of operations to achieve desired time, temperature and shear history for the processed materials. These advantages overcome many of the problems that occur in single-screw extruders such as the poor mixing ability, the inability to transport sticky and gummy materials and the limited range of raw material particle sizes. However, single-screw extruders are useful in many food applications because they are mechanically simpler, easier to operate and less expensive than twin-screw extruders (Frame, 1994; Gogoi, 1996; Riaz, 2000).

4. Extrusion Parameters

Extrusion is a powerful food processing operation with versatility to produce a wide variety of food products with unique sensory properties. Small variations in process parameters can greatly influence product quality (Desrumaux *et al*., 1999).

Process parameters that can be varied to impact desired product characteristics include screw speed, barrel temperature, feed rate, moisture content, composition of feed, die configuration, screw configuration and others specific to the product. System parameters such as specific mechanical energy, die pressure, residence time as well as target parameters including expansion ratio, water solubility index, water absorption index, and bulk density can be varied to achieve the desired sensory properties (Chen *et al*., 1991; Gogoi *et al*., 1996; Guha *et al*., 1997). The interactions among the processing parameters are complex and influence the changes in the final product (Chen *et al*, 1991). Effects of the process variables on final product characteristics are product and machine specific and conclusions cannot extend beyond the scope of the research (Guha *et al*., 2003). The effects of process variables and others parameters to product quality are shown in Figure 3.

n-3 Fatty Acids

1. n-3 Fatty Acids

n-3 Fatty acids are long chain polyunsaturated fatty acids (PUFAs) in which the first double bond is located three carbon atoms from the methyl end (Stone, 1997, IOM, 2002). They are α-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3), docosapentaenoic acid (DPA; C22:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). EPA and DHA are occasionally referred as timnodonic acid and nervonic acid (Nettleton, 1995) and, generally, as the fish oil fatty acids.

α-Linolenic acid is considered to be essential in the human's diet because it cannot be synthesized and lack in the diet produces clinical symptoms including neurological problems and poor growth (Connor, 2000; IOM, 2002). Differences in biosynthesis of long-chain fatty acids are shown in Figure 4. α-Linolenic acid is the precursor of EPA and DHA, and humans require a dietary source of n-3 fatty acids due to lack of ability to insert a double bond at the n-3 position of a C_{18} fatty acid (IOM, 2000). Additionally, n-3 fatty acids cannot be synthesized from saturated, n-9 monounsaturated or n-6 PUFA (IOM, 2000). Therefore, ALA is considered the

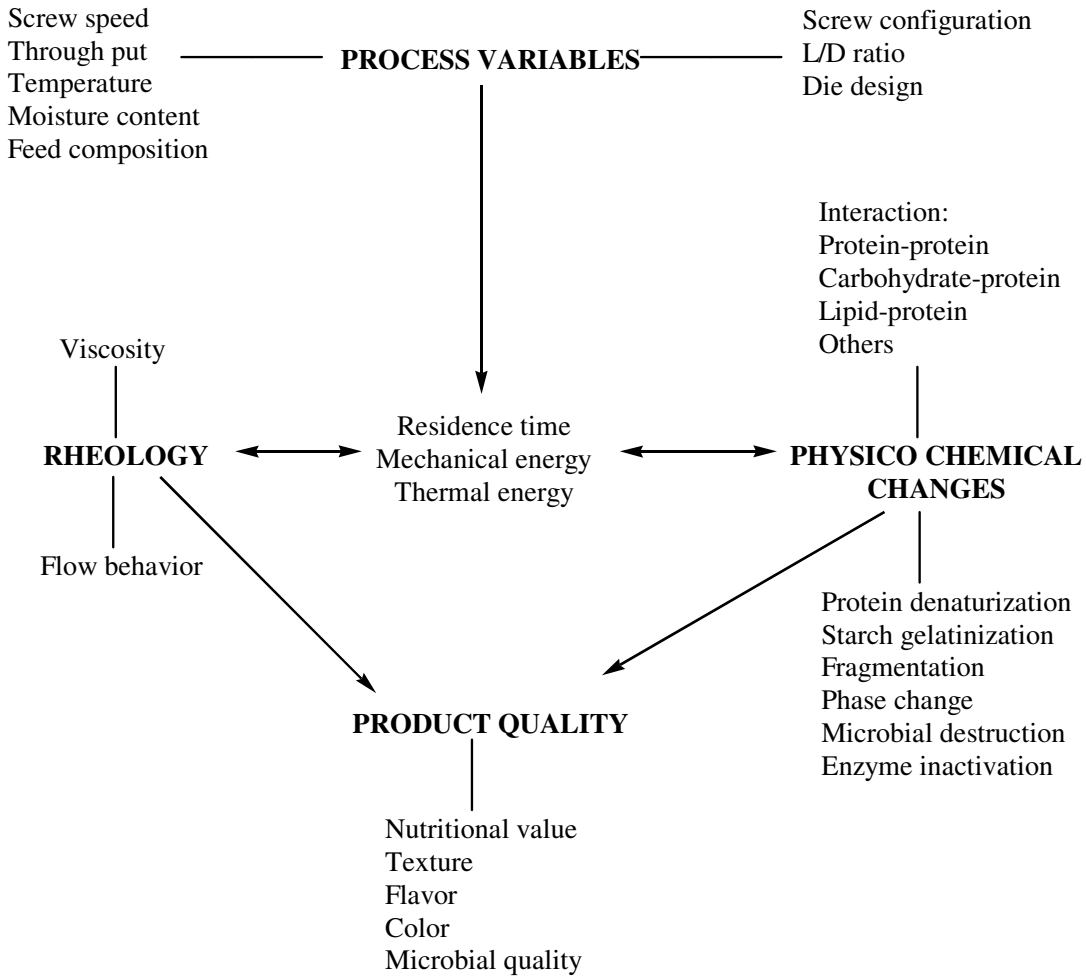


Figure 3 Schematic of extrusion processing illustrating the effects of process variables on product attributes

Source: Choudhury and Gautam (1999)

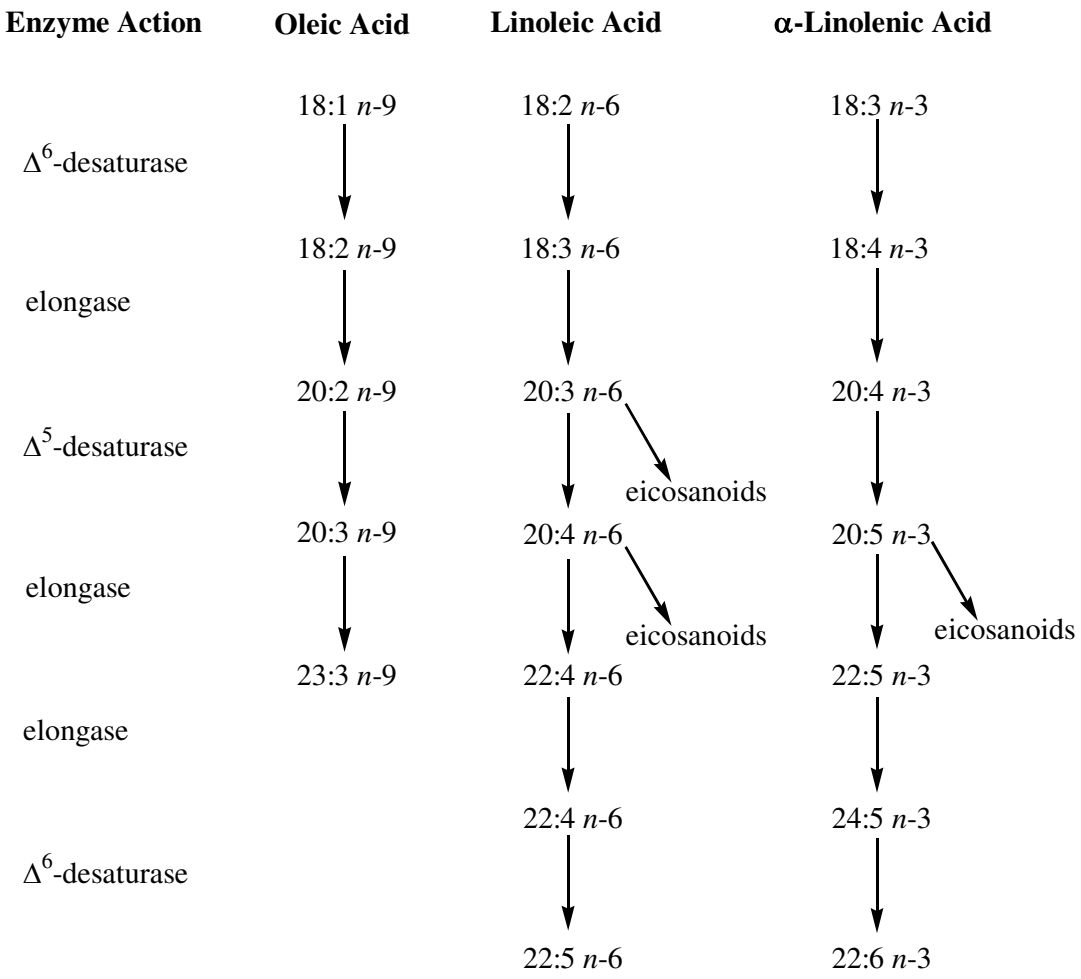


Figure 4 Biosynthesis of long-chain fatty acids

parent n-3 fatty acid. It is elongated and desaturated to longer chain and more unsaturated forms by the same system active on n-6 fatty acids (Figure 4). Desaturation by Δ6 desaturase and elongation and further desaturation by Δ5 desaturase forms EPA, which serves as the source of the series 3 eicosanoids and series 5 leukotrienes. Addition of two 2-carbon units and action of 6 desaturase with β-oxidation forms DHA from 24:5n-3 (Figure 4).

α-Linolenic acid is primarily from plant sources (FDA/ CFSAN, 2000; Kris-Etherton *et al*., 2000, 2002). Eicosapentaenoic acid and DHA in seafood originate from marine phytoplankton and algae. They are transferred to marine animal and seafood species through the food web. Therefore, the consumption of marine products will increase human intake of n-3 fatty acids (Shahidi and Wanasundara, 1998).

Significant research has been carried out on the metabolism of PUFA in general and n-3 fatty acids in particular. n-3 Fatty acids are essential for normal growth and development and may play an important role in prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, inflammatory and autoimmune disorders, and cancer (Simopoulos, 2000). n-3 Fatty acids are significant structural components of phospholipid membranes throughout the body. They are found in high concentrations in retina, brain and spermatozoa (Connor, 2000).

2. Sources of n-3 Fatty Acids

Marine fish are the major sources of EPA and DHA; whereas, vegetable oil is the major source of ALA. Nuts, seeds, some vegetables and fruits, egg yolk, poultry and meat are minor sources of n-3 fatty acid (Kris-Etherton *et al.*, 2000, 2002). Tofu, soybean, canola oil and nuts are important plant sources of n-3 fatty acids for vegetarian and non-seafood eaters (Stone, 1997). All fish contain EPA and DHA; however, the quantities vary among the species and within the species according to diet and environment (Kris-Etherton *et al*., 2002). High fat species such as tuna, salmon, mackerel, herring, and sardines have higher quantities of n-3 fatty acids

associated with the muscle. Fatty acids compositions of fish vary mainly by diet. Others factors that influence fatty acids compositions are size or age, reproductive status, geographical location and season. Cold water fish usually accumulate higher amounts of fat and n-3 fatty acids as a protective mechanism against cold temperature. The relative amounts of EPA and DHA vary in fish species and DHA is more abundant than EPA in most species. By contrast, refined fish oils always have more EPA than DHA (Nettleton, 1995). Table 1 presents n-3 fatty acid contents of fish shellfish and fish oils derived from the USDA Nutrient Database (USDA, 2004).

Dickinson (2002) stated that eating more fish is an excellent way to increase the consumption of n-3 fatty acids. However, because fish consumption is low in many countries, another way is to use dietary supplements. There are many available products in the markets that are enriched with n-3 fatty acids, for example, oils in capsule form, bakery products, eggs, infant formula, milk, mayonnaise, meat and poultry products, and intensively cultured fish such as salmon that have increased n-3 fatty acid content (Simopoulos, 2000).

3. Importance of n-3 Fatty Acids in Health and Disease

n-3 and n-6 Fatty acids are precursors of hormone like substances, known as eicosanoids, which are involved in many important biological processes in the human body. The imbalances of n-3 and n-6 fatty acids are believed to be the causes of diseases such as cardiovascular disease, hypertension, inflammatory and autoimmune disorders, depression and certain disrupted neurological functions (Shahidi and Wanasundara, 1998). Substitution of n-3 for n-6 fatty acids in the metabolic system of eicosanoids initiates the formation of prostaglandins and thromboxanes that have lowered biological activity (Arkhipenko and Sazontova, 1995) (Figure 5 and 6). Connor (2000) categorized the nutritional significance of n-3 fatty acids into the following categories:

a. Significant structural components of phospholipids associated with membrane structure

Table 1 n-3 Fatty acid content of fish, shellfish and fish oil

	n-3 Fatty Acids (g/100g)	
	EPA	DHA
Catfish		
Wild, raw	0.130	0.234
cooked, dry heat	0.100	0.137
Farmed, raw	0.067	0.207
cooked, dry heat	0.049	0.128
Cod		
Pacific, raw	0.080	0.135
cooked	0.103	0.173
Atlantic, raw	0.064	0.120
cooked, dry heat	0.004	0.154
canned, solid and liquid	0.004	0.153
dried and salted	0.011	0.423
Flounder (flounder and sole)		
raw	0.093	0.106
cooked, dry heat	0.243	0.258
Mackerel		
King, raw	0.136	0.177
cooked, dry heat	0.174	0.227
Spanish, raw	0.329	1.012
cooked, dry heat	0.294	0.952
Jack, canned, drained solids	0.434	0.796
Pacific and Jack mixed species, raw	0.509	0.932
cooked	0.653	1.195

Table 1 (continued)

	n-3 Fatty Acids (g/100g)	
	EPA	DHA
Tuna		
Skipjack, raw	0.071	0.185
Yellow fin, raw	0.037	0.181
cooked, dry heat	0.047	0.232
Bluefin, raw	0.283	0.890
Canned tuna		
Light, canned in water, drained solids	0.047	0.223
White, canned in water, drained solids	0.233	0.629
Salmon		
Pink, raw	0.419	0.586
cooked, dry heat	0.537	0.751
canned, solids with bone and liquid	0.845	0.806
Chum, raw	0.233	0.394
cooked, dry heat	0.299	0.505
canned, without salt, drained solids, with bone	0.473	0.702
Shrimp, mixed species, raw	0.258	0.222
cooked, moist heat	0.171	0.141
canned, moist heat	0.293	0.025
Crab		
Alaska king crab, raw	0.130	
cooked, moist heat	0.295	0.118
Blue, raw	0.170	0.150
cooked, moist heat	0.243	0.231
canned	0.193	0.170

Table 1 (continued)

	n-3 Fatty Acids (g/100g)	
	EPA	DHA
Scallop, mixed species, raw	0.090	0.108
cooked	0.166	0.199
Fish Oil		
Cod liver	0.898	10.968
Herring	6.273	4.206
Menhaden	13.168	8.562
Salmon	13.023	18.232
Sardine	10.137	10.656

Source: USDA National Nutrient Database for Standard Reference, Release 16-1 (2004)

b. Significant to the prevention and/or modulation of various diseases including coronary heart disease, stroke, essential fatty acid deficiency in infants, autoimmune disorders, Crohn's disease, various cancers (breast, colon, prostate), hypertension and rheumatoid arthritis.

c. α-linolenic acid (ALA) is essential and must be supplied in the diet.

Associations of n-3 fatty acid intake to prevention and/or modulation of chronic diseases have led to a large body of scientific research. In the following section, current information is provided about the impact of n-3 fatty acid intake on various disease states.

3.1 n-3 Fatty Acids and Coronary Heart Disease (CHD)

In 2000, the U.S. Food and Drug Administration (FDA) issued a qualified health claim for use of n-3 fatty acids in supplements in relation to coronary heart disease (CHD). FDA evaluated all of the clinical evidence available up to 2000 and concluded that a sufficient basis existed to support the qualified health claim on intake of n-3 fatty acids and reduced incidence of CHD (FDA/CFSAN, 2000). Although FDA issued the qualified health claim, it was stated that significant scientific agreement did not exist among experts to support a general health claim for conventional foods stating that consumption of n-3 fatty acids may reduce the risk of CHD for the general population.

In a letter clarifying conditions for a dietary supplement health claim for n-3 fatty acids and CHD, FDA provided the following satisfactory qualified claim (FDA/CFSAN, 2002): "It is known that diets low in saturated fat and cholesterol may reduce the risk of heart disease. The scientific evidence about whether omega-3 fatty acids may reduce the risk of coronary heart disease (CHD) is suggestive, but not conclusive. Studies in the general population have looked at diets containing fish and it is not known whether diets or omega-3 fatty acids in fish may have a possible effect

on reduced risk of CHD. It is not known what effect omega-3 fatty acids may or may not have on risk of CHD in the general population".

FDA has stated, through issuance of the qualified health claim for supplements, that use of EPA and DHA as dietary supplements is safe and lawful provided that the daily intakes of EPA and DHA from the diet and use of supplements does not exceed an intake of 3g/d. In order to help ensure that a consumer does not exceed an intake of 3g/d, EPA and DHA n-3 fatty acid supplements labeled with the qualified health claim should not recommend or suggest in labeling, or under ordinary conditions of use, a daily intake greater than 2g of EPA plus DHA directly from the supplement. The qualified health claim recently was approved for use on conventional food labels that contain EPA and DHA (FDA, 2004).

In 2002, the American Heart Association (AHA) issued a scientific statement on fish consumption, fish oil, n-3 fatty acids and cardiovascular disease (CVD) (Kris-Etherton *et al*., 2002; 2003). The AHA conclusion was that n-3 fatty acids have been shown through epidemiological and clinical studies to reduce the incidence of CVD. Evidence suggested that EPA+DHA obtained through the diet or supplements at 0.5 to 1.8g/d significantly reduced cardiac and all-cause mortality. α-Linolenic acid intakes of 1.5 to 3.0g/d seemed beneficial. American Heart Association Dietary Guidelines recommend at least two servings of fish per week and the use of vegetable oils high in ALA for the general population. The dietary recommendations must be balanced with potential intake of environmental pollutants including polychlorinated biphenyls (PCBs), dioxins and methylmercury taken into consideration.

Connor (2000) stated that mechanisms by which n-3 fatty acids may reduce CVD include reduced susceptibility to ventricular arrhythmia, antithrombogenic effects, hypotriglyceridemic effects, retardation of atherosclerotic plaque development through reduced adhesion molecule expression, reduced platelet-derived growth factor expression and general anti-inflammatory effects, promotion of

nitric oxide induced endothelial reactions and hypotensive effects. The AHA made the following summarizations:

Triglycerides – Hypotriglyceridemic effects of n-3 fatty acids are well established. A dose-response relationship exists between n-3 fatty acid intake and triglyceride lowering. Small intakes less than 2 g/d produce significant reductions. Protective levels of intake against CVD can be lower than 1 g/day.

Blood pressure – n-3 Fatty acids have a dose-dependent hypotensiveeffect. Docosahexaenoic acid seems to be more effective than EPA in lowering blood pressure. However, an increased intake of n-3 fatty acids has only a limited role in the management of hypertension due to the high dosage level required for impact.

Thrombosis and hemostatis – Thrombosis is the formation of a blood clot in tissues and blood vessels. Blood clot or thrombus can completely block blood vessels. When it occurs in heart or brain, the result is heart attack and stroke, respectfully (Nettleton, 1995). Hemostatis is the process of the formation and degradation of blood clots that allow the body to control blood loss and protect against stroke (Lefevre *et al*., 2003). Intake of n-3 fatty acids can beneficially affect platelet aggregation and hemostasis. Effects on thrombosis are inconclusive.

Arrhythmias – n-3 Fatty acids may reduce the risk for sudden cardiac death induced by arrhythemias. Mechanisms include prevention of calcium overload by maintaining activity of L-type calcium channels during stress and by increasing cardia Ca^{+2}/Mg^{+2} ATPase activity.

The AHA recommendations for n-3 fatty acid intake are the following:

Patients without documented CHD – Eat a variety (preferably oily) of fish at least twice a week. Include oils and foods rich in ALA (flaxseed, canola, soybean oils; flaxseed and walnuts).

Patients with documented CHD – Consume approximately 1g of EPA+DHA per day, preferably from oily fish. EPA+DHA supplements could be considered in consultation with the physician.

Patients needing triglyceride lowering – Two to four g of EPA+DHA per day provided by capsules under a physician's care.

Recent reviews (De Caterina *et al*., 2004; Din *et al*., 2004; Lefevre *et al*., 2004; Weisman *et al*., 2004) clearly conclude that scientific literature supports the view that n-3 fatty acids from fish or fish oils can protect the human from CHD. The reviews present the AHA guidelines as the preferred recommendations for beneficial impact of n-3 fatty acids on health. Recent research presents the following conclusions:

1.) Among women, higher consumption of fish and n-3 fatty acids is associated with a lower risk of CHD, particularly CHD deaths (Hu *et al*., 2002).

2.) A higher consumption of fish and n-3 fatty acids is associated with a lower CHD incidence and total mortality among diabetic women (Hu *et al*, 2002).

3.) Higher proportions of n-3 fatty acids in serum lipids are associated with a substantially reduced risk of death (Erkkilä, *et al*., 2003).

4.) Fish consumption is associated with a significantly lower risk of fatal and total CHD. The conclusion was based on a meta-analysis of 19 observational studies published since 1985 (Whelton *et al*., 2004)

3.2 n-3 Fatty Acids and Stroke

Due to the ability to inhibit platelet aggregation, intake of n-3 fatty acids has been associated with incidence of stroke (Kris-Etherton *et al*., 2002; He *et al*., 2002). Associations have been made between fish consumption and reduction of risk

of ischemic stroke and increased risk of hemorrhagic stroke (He *et al*, 2002). However, the literature is not conclusive. Iso *et al*. (2001) in the Nurses' Health Study involving 79,839 women over a 14 years period found a significant inverse association between fish intake and risk of ischemic (thrombotic) stroke but not for hemorrhagic stroke. Women in the highest quintile of n-3 fatty acid intake had reduced risk of total stroke and ischemic stroke. No association was apparent between intake of n-3 fatty acids and hemorrhagic stoke. Iso *et al.* (2001) concluded that consumption of fish and n-3 fatty acids was not related to hemorrhagic stroke and that regular consumption of fish may be beneficial for prevention of thrombotic infarction in middle-aged women in the United States.

He *et al*. (2002), reported on the Health Professional Follow-up Study that followed 43,671 men for 12 years who were free of cardiovascular disease at the start of the study. The data indicated that eating fish once per month reduced the risk of ischemic (thrombotic) stroke in men. This study showed no significant associations of fish or n-3 fatty acid intake and risk of hemorrhagic stroke. Prior work with 2,828 patients with coronary heart disease who received a supplement of 1g n-3 fatty acid per day indicated that the high level supplementation decreased the risk of cardiovascular death, non-fatal myocardial infarction and stroke measured as a combined endpoint (GISSI-Prevenzione Investigation, 1999).

Caicoya (2002) studied 440 stroke patients and 473 controls and related fish consumption to risk of stroke. Risk of stroke increased with consumption of fish. Individuals in the highest quintile of n-3 fatty acid consumption (660 mg/d) were at borderline higher risk of stroke as compared to individuals in the lower quintile of n-3 fatty acid consumption (115 mg/day).

A recent study by Skerrett and Hennekens (2003) reviewed past research and related consumption of fish and fish oil to risk of stroke. These authors concluded that data support the hypothesis that consumption of fish several times per week reduces the risk of thrombotic stroke but does not increase the risk of hemorrhagic stoke. Kris-Etherton *et al*. (2002) maintain that relatively little

information is available associating n-3 fatty acid intake and stroke. Final conclusions are yet to be made.

3.3 n-3 Fatty Acids and Cancer

Terry *et al*. (2001) reviewed available epidemiologic studies on the relationship between intake of n-3 fatty acids and the risks of various cancers including breast and prostate cancers. The review included 52 cohort and case-control studies relevant to fish and n-3 fatty acid intake and risk of breast and prostate cancer. Conclusions reached by the authors include the following:

"1.) The development and progression of breast and prostate cancers appear to be affected by processes in which EPA and DHA play important roles; yet, whether the consumption of fish containing marine fatty acids can alter the risk of these cancers or of other hormone-dependent cancers is unclear.

2.) There are still too few data from epidemiologic studies to evaluate the strength, consistency, and dose response of the relation between marine fatty acid intake and human cancer.

3.) Although there is ample evidence from *in vitro* and animal studies that these essential fats can inhibit the progression of tumors in various organs, particularly breast and prostate, the evidence from epidemiologic studies is less clear.

4.) The recommendation of the American Heart Association (AHA, 2003) to eat 2 servings of fish/ week, especially fatty fish for the prevention of sudden cardiac death, may have additional benefits, including those related to blood triacylglycerol concentration, clotting mechanisms, blood pressure, the immune system, and the developing central nervous system. The potential benefits of an increased intake of marine fatty acids with respect to cancer prevention have yet to be established clearly, but they may be important."

Larsson *et al*. (2004) stated in a review of literature on potential mechanisms of n-3 fatty acids for the prevention of cancer that "increasing evidence from animal and *in vitro* studies indicates that n-3 fatty acids, especially long-chain polyunsaturated fatty acids EPA and DHA, present in fatty fish and fish oil inhibit carcinogenesis". Potential anticarcinogenic mechanisms of n-3 fatty acids presented by Larsson *et al*. (2004) include the following:

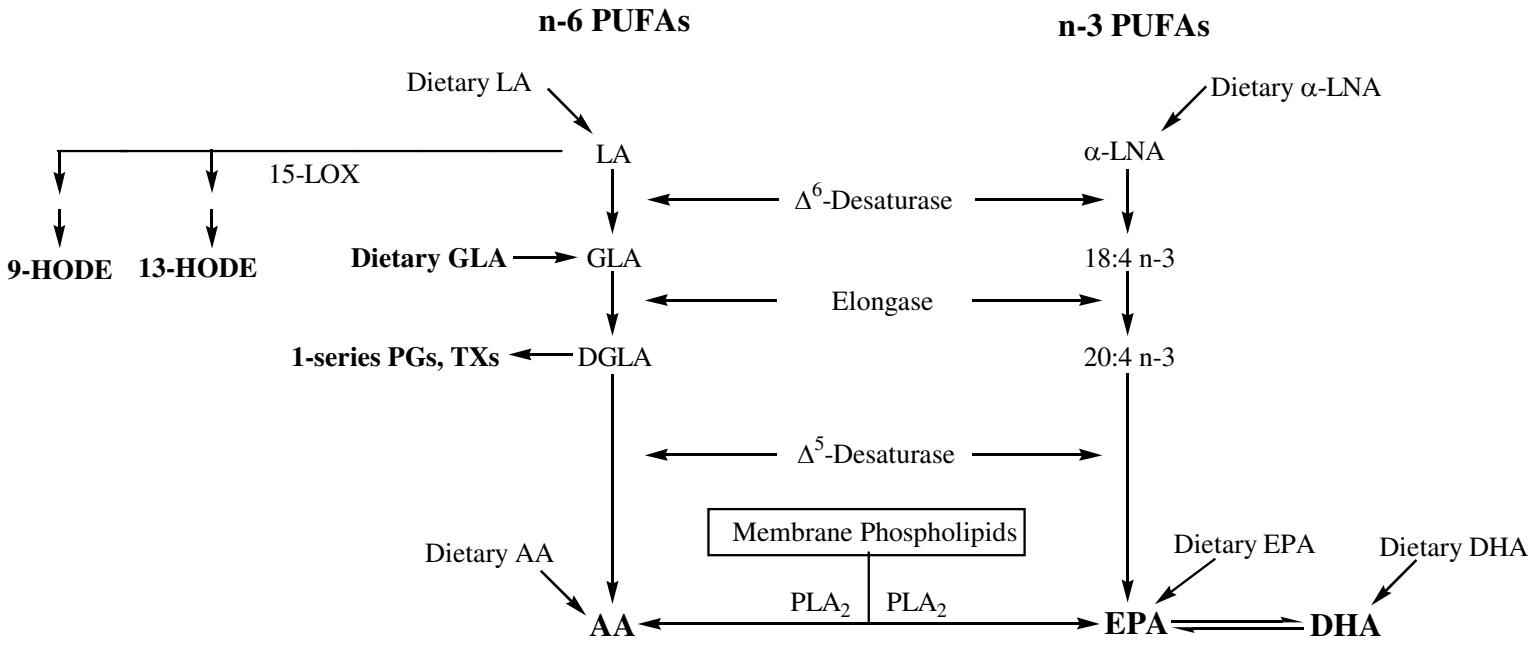
a. n-3 Fatty acids may lower the risk of cancer by their suppressing effect on the biosynthesis of arachidonic acid (AA, 20:4n-6)-derived eicosanoids (Figures 5 and 6). Eicosanoids are biologically significant, hormone-like lipids containing 20 carbons. They modulate inflammatory and immune responses and impact platelet aggregation, cell growth and cell differentiation. Precursors in their synthesis are dihomo-γ-linolenic acid (DGLA, 20:3n-6), AA and EPA (Figure 5). Linoleic acid (LA, 18:2n-6) and linolenic acid (α-LNA) are plant origin precursors of DGLA and AA and EPA, respectfully (Figure 5).

The suppressing effect of n-3 fatty acids on AA-derived eicosanoids was summarized by Larsson *et al*. (2004) to include the following aspects:

1.) High intake of n-3 fatty acids results in higher n-3 fatty acid levels in membrane phospholipids, replacing AA

2.) A decrease in the availability of AA precursors suppresses the synthesis of AA-derived eicosanoids and increases EPA-derived 3-series prostanoids and 5-series leukotrienes.

3.) n-3 Fatty acids compete with n-6 PUFAs for desaturases and elongases. Since the n-3 fatty acids have greater affinities for the enzymes, a higher intake of n-3 fatty acids decreases conversion of LA and AA (Figure 5) and the production of AA derived eicosanoids (Figure 6).

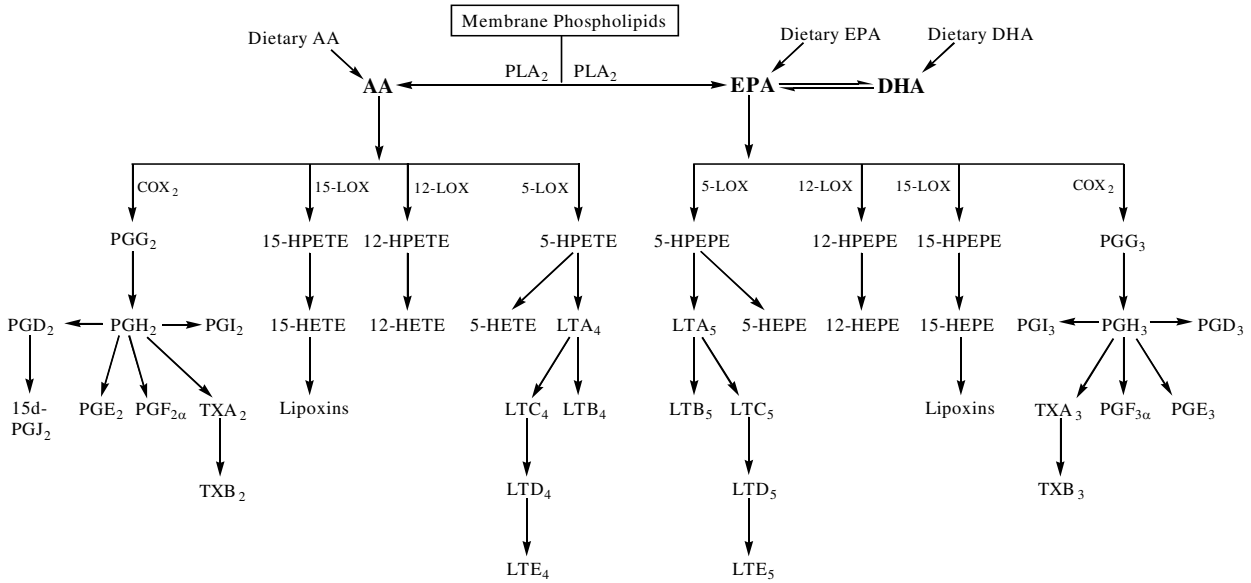


LA = linoleic acid (18:2n-6)
α-LNA = α-linolenic acid (18:3n-3)
GLA = γ-linolenic acid (18:3n-6)
DGLA = dihomo-γ-linolenic acid (20:3n-6)
AA = arachidonic acid (20:4n-6)

EPA = eicosapentaenoic acid (20:5n-3)
DHA = docosahexaenoic acid (22:6n-3)
PLA_2 = phospholipase A_2
LOX = lipoxygenase
COXs = cycloxygenase (COX-1 and COX-2)

Figure 5 Biosynthesis of eicosanoids from n-6 fatty acids and formation of AA, EPA and DHA

Source: Lasson *et al.* (2004)



LA = linoleic acid (18:2n-6)
α-LNA = α-linolenic acid (18:3n-3)
GLA = γ-linolenic acid (18:3n-6)
DGLA = dihomo-γ-linolenic acid (20:3n-6)
AA = arachidonic acid (20:4n-6)
EPA = eicosapentaenoic acid (22:5n-3)
DHA = docosahexaenoic acid

PLA_2 = phospholipase A_2
LOX = lipoxygenase
COXs = cycloxygenase (COX-1 and COX-2)
HPEPE = hydroxyeicosapentaenoic acid
15-HETE = 15(*S*)-hydroxyeicosatetraenoic acid
12-HETE = 12-hydroxyeicosatetraenoic acid
5-HETE = 5-hydroxyeicosatetraenoic acid

HEPE = hydroxyeicosapentaenoic acid
HPETE = hydroperoxyeicosatetraenoic acid
LT = leukotriene
HODE = hydroxyoctadecadienoic acid
PG = prostaglandin
TX = Thromboxane

Figure 6 Conversion of AA, EPA and DHA to prostagladins, thromboxanes, and leukotriene eicosanoids

Source: Lasson *et al.* (2004)

4.) Lipoxygenases have more affinity for EPA compared toAA. Therefore, increased levels of EPA lead to greater formation of EPA-derived lipoxygenase products and lower amounts of AA-derived lipoxygenase products (Figure 6).

5.) Formation of AA-derived eicosanoids is decreased by n-3 fatty acids and, additionally, which in some cases are more inhibitory than EPA.

6.) n-3 Fatty acids increase eicosanoid metabolism, possibly by induction of peroxisomal enzymes, which include enzymes required for β-oxidation of fatty acids.

7.) Combined, the above effects reduce the synthesis of AA-derived eicosanoids which are involved with anti-inflammatory processes and carcinogenesis.

b. n-3 Fatty acids and other PUFAs may affect gene expression and the activities of signal transduction molecules involved in the control of cell growth, cell differentiation, apoptosis, angiogenesis and metastasis.

c. A high intake of n-3 fatty acids relative to n-6 PUFAs may decrease estrogen production. Estrogen has effects on cell proliferations on estrogen sensitive tissues, and high estrogen levels may increase the risk of breast cancer and other hormone-dependent cancers.

d. Inflammation is theorized to increase the production of free radicals and reactive oxygen species, which leads to carcinogenesis. n-6 Fatty acids increase production of AA-derived proinflammatory eicosanoids; whereas, n-3 fatty acids suppress inflammation and the increased production of free radicals and carcinogenesis.

Various studies suggest that risk of prostate cancer is reduced with increased intake of EPA and DHA. Recent studies include those by Norrish *et al*. (1999), Terry *et al*. (2001) and Augustsson *et al*. (2003). Norrish *et al*. (1999) studied 317 prostate cancer patients and 480 age-matched community controls in New Zealand. The study associated reduced risk of prostate cancer with increased levels of EPA and DHA in erythrocytes. Only moderated correlations were found between erythrocyte levels of EPA and DHA and self-reported fish intake. Terry *et al*. (2001) studied the association between high fatty fish consumption and prostate cancer in 6272 men in Sweden. The study followed the men for 30 years and showed that men who ate no fish had a two to three fold higher frequency of prostate cancer compared to men who ate moderate to high amounts of fish. The results support the belief that consumption of fatty fish and, thus, higher levels of n-3 fatty acids, lowers the risk of prostate cancer. Augustsson *et al*. (2003) followed 47,882 subjects in the Health Professionals Follow-up Study since 1986. Eating fish more than 3 times/week was associated with a lower risk of prostate cancer. A lower association was found for intake of n-3 fatty acids. Use of fish oil supplement was not associated with a decreased risk of cancer.

3.4 n-3 Fatty Acids and Inflammation and Autoimmune Diseases

3.4.1 n-3 Fatty Acids and Inflammation

Recent reviews summarize the large number of research articles covering the relationship of n-3 fatty acid intake to inflammation and autoimmune disease onset and progression (Ergas *et al*., 2002; Simopoulos, 2002; Clevland *et al*., 2003). Table 2 summarizes effects of n-3 fatty acids on factors involved in inflammatory processes. Simopoulos (2002) characterized the significance of n-3 fatty acid intake to inflammatory processes as follows:

1.) n-6 Fatty acids predominate in western diets. n-6 and n-3 Fatty acids are metabolically distinct and have opposing physiological functions.

2.) Eicosapentaenoic acid (EPA) competes with AA and induces the synthesis of less inflammatory derivatives.

3.) Animal and human studies indicate that n-3 fatty acids suppress cell mediated immune responses.

4.) The increased n-6/n-3 ratio in western diets most likely contributes to increased inflammatory disorders and cardiovascular disease.

5.) Elevated levels of cytokines are usually noted in patients with autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease (Crohn's disease) and asthma. Supplementation of the diet with EPA and DHA decreases the elevated cytokine levels.

Although the magnitude of research relating n-3 fatty acid intake to decreased inflammatory responses is too large to discuss individual research papers in this literature review, an example of such work is provided by Adam *et al*. (2003). These authors studied the anti-inflammatory effects of a low AA diet and fish oil supplementation on patients with rheumatoid arthritis. A normal western diet (WD) was compared to a low AA diet considered anti-inflammatory (AID diet) and providing less than 90 mg AA/day. Patients in both groups received 30mg/kg body weight/day of placebo or fish oil capsules. The study concluded that a diet low in AA ameliorates clinical signs of inflammation in patients with rheumatoid arthritis and augments the beneficial effects of diet supplementation with fish oil. Specific effects noted in the study included:

1.) In the AID but not in the WD, the number of tender and swollen joints decreased by 14% during placebo treatment.

2.) In AID compared to WD, fish oil supplementation led to a significant reduction in the number of tender (28% *vs* 11%) and swollen (34% *vs* 22%) joints ($p<0.01$).

Table 2 Effects of n-3 fatty acids on factors involved with inflammation

Effect	Response Factor	Function
Decreased by n-3 fatty acids	Arachidonic acid	Eicosanoid precursor, aggregates platelets, stimulates white blood cells
	Thromboxane	Aggregates platelets, vasoconstriction, increases intracellular Ca^{++}
	Fibrinogen	Factor in acute phase response, clotting factor
	Leukotriene (LTB_4)	Neutrophil chemoattractant, increases intracellular Ca^{++}
	Platelet activating factor (PAF)	Activates platelets and white blood cells
	Platelets-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages
	Oxygen free radicals	Cellular damage, enhances LDL uptake via scavenger pathway, stimulates AA metabolism
	Lipid hydroperoxides	Stimulates eicosanoid formation
	Interleukin 1 and tumor necrosis factor	Stimulates neutrophil O_2 free radical formation, stimulates lymphocyte proliferation, stimulates PAF, expression of intracellular adhesion melecule-1 and inhibits plasminogen activator, therefore, acts as a procoagulant
	Interleukin-6	Stimulates C-reactive protein, serum amyloid A, fibrinogen. α_1-chymotrypsin and haptoglobin (acute phase proteins involved in the inflammation process)
Increased by n-3 fatty acids	Prostacyclin ($PGI_{2/3}$)	Prevents platelet aggregation, vasodilator, increases camp
	Tissue plasminogen activator	Increases endogeneous fibrinolysis

Modified from Simopoulos (2002)

3.) Fish oil supplementation of the AID increased EPA in erythrocyte lipids by 244%, lowered formation of leukotriene B_4 by 34%, lowered levels of 11-dehydro-thromboxane B_2 by 15% and decreased prostaglandin metabolites by 21%. All of these changes were greater than when fish oil was supplemented into WD.

4. n-3 Fatty Acids Supplementation

n-3 Fatty acids, especially EPA and DHA, have positive influence on human health as well as other animals. The intakes of these fatty acids are usually low, therefore the increased consumption of n-3 fatty acids is recommended (Kolanowski *et al*., 1999). When the n-3 fatty acids in conventional food is not enough, the n-3 supplement products may be recommended to substitute or supplement the dietary lipids. The fish oil and fatty acid supplements were the best seller among food supplement products in year 2002 (Anon, 2002). Marine oil supplements are currently available as non-prescription in United States, European countries and Canada. Most of the marine oil products have not been structurally altered and are in triaclglycerols (TAG) form. Products in the free fatty acid, methyl and ethyl ester concentrate forms are being sold. Concentrates n-3 fatty acids are preferred items for pharmaceutical application as well as possible enrichment of foods (Shahidi and Wanasundara, 1998). Fish oil and fatty acids were the one of the top selling supplement in 2002 with the highest sales growth of 58% (NMI, 2002). A number of food product enriched with n-3 fatty acids have been launched over years. In 2001, 174 new products with n-3 fatty acid were launched worldwide, of which 29% were pet foods, 24% were dietary supplement, 15% were dairy foods, 14% were processed fish, meat and eggs product, 3% were snack and 2% were baby food, bakery and beverage (Mintel's Global New Products Database, 2001; Anon, 2002)

Criteria that must be met for the successful incorporation of PUFA into food include bioavailability and stability to processing and storage. The incorporation of PUFA as an ingredient should not change the sensory properties of the food (Augustin and Sanguansri, 2003).

5. Stability of n-3 Fatty Acids

Because of the high level of unsaturation, incorporation of fish oil into food products can lead to rapid oxidative deterioration of unsaturated fatty acids with production of off-odors and off-flavors. Use of antioxidants to retard oxidation in fish oil or products containing fish oil has been studied extensively because of the susceptibility of the PUFA, including EPA and DHA, to oxidation.

Kamal-Eldin and Yanishlieva (2002) reviewed the factors significant to stability of n-3 fatty acids in food systems. These authors stated that the n-3 PUFA are difficult to stabilize against lipid oxidation and that use of natural antioxidants is not a simple answer for stabilization. In general, all factors that can initiate lipid oxidation are significant to the stability of EPA and DHA because of their inherent high susceptibility to oxidation compared to less unsaturated fatty acids. Kamal-Eldin and Yanishlieva (2002) stressed that optimal stabilization of n-3 fatty acids includes use of antioxidants together with minimal exposure to air, light, metal contamination and high temperatures during processing and storage. Most research has been carried out on the heat stability of PUFA oils which can be expected to be different to compared the stability of PUFA in food systems. Therefore, lipid oxidation in multi-phase food systems is not well understood (Augustin and Sanguansri, 2003).

Many studies have been completed on the antioxidant activity of natural tocopherols in fish oil. Kulås and Ackman (2001a, b, c) and Kulås *et al*. (2002) studied the relative antioxidant activity of α-, γ- and δ-tocopherols. At 100 ppm (0.01%) concentration, the relative ability the tocopherols to inhibit oxidation in purified fish oils decreased in the order α-T > γ-T > δ-T. A reverse order of antioxidant activity was noted at 1000 ppp (0.1%). None of the tocopherols were prooxidant at 1000 ppm. At 100 ppm, the same order of activity (α-T > γ-T > δ-T) was seen in the ability of the tocopherols to reduce formation of volatiles in the oils (Kulås *et al*., 2002).

Kulås and Ackman (2001c) also noted that the presence of minor components in non-purified menhaden oil affected oxidation. Formation of hydroperoxides occurred more rapidly in the non-purified oil compared to the purified oil. Hamilton, *et al*. (1998) showed that δ-T alone or in combination with lecithin and ascorbyl palmitate were effective antioxidants in delaying hydroperoxide formation in refined menhaden or anchovy oils. The most effective antioxidant mixture was a ternary mixture of 2% δ-T, 0.1% ascorbyl palmitate and 0.5% lecithin (wt % in the oil). This antioxidant mixture delayed oxidation at 20°C for 6 months. A recent study determined the effects of light, temperature and synthetic antioxidants on stability of mackerel liver oil (Sang and Jin, 2004). Visible light exposure was the most important factor leading to oxidation. The oxidation rate of the mackerel liver oil decreased in the following order: control with light > control + BHA with light > control without light > control + TBHQ with light > control + BHA without light > control + TBHQ without light. An increase in storage temperature from 5°C to 40°C, increased the rate of oxidation only in the presence of light. The superior effect of TBHQ compared to other synthetic antioxidants in fish oil was previously shown by Kaitaranta (1992). Sang and Jin (2004) concluded that avoidance of light exposure, addition of antioxidants and low storage temperatures can contribute alone or in combination to prevent oxidation in fish oil products.

Microencapsulation has been successfully demonstrated to improve stability of fish oil by ten fold (Baik *et al*., 2004). Addition of α-T (> 200 ppm) further improved stability. Relative few studies are available considering the effectiveness of antioxidants on n-3 fatty acid in food products other than fish oil. Park *et al*. (2004) showed that n-3 fatty acids added to surimi when the fish oil was fortified with 1000 ppm rosemary extract and 500 ppm ascorbyl palmitate prior to incorporation into the surimi.

No information is available to specifically show the effects of extrusion on n-3 fatty acid content. However, mixed tocopherols were shown to have lower stability during extrusion of a fish snack compared to extrusion of a peanut snack (Suknark *et*

al., 2001). These authors postulated that the tocopherols were decreased to a greater extent in the fish snack due to their increased antioxidant action to protect the higher amount of PUFA in the fish containing extrudate compared to the peanut formulation.

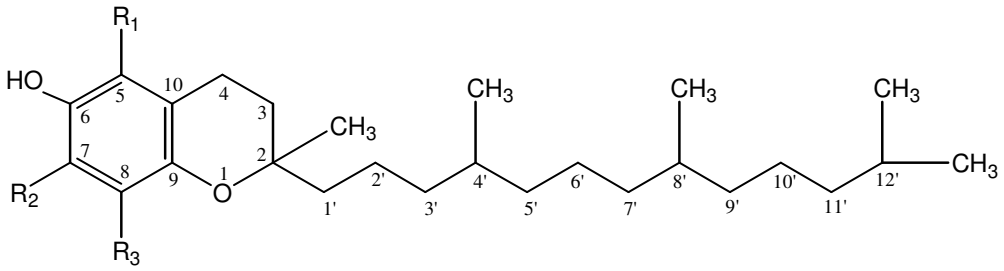
Vitamin E

1. Vitamin E

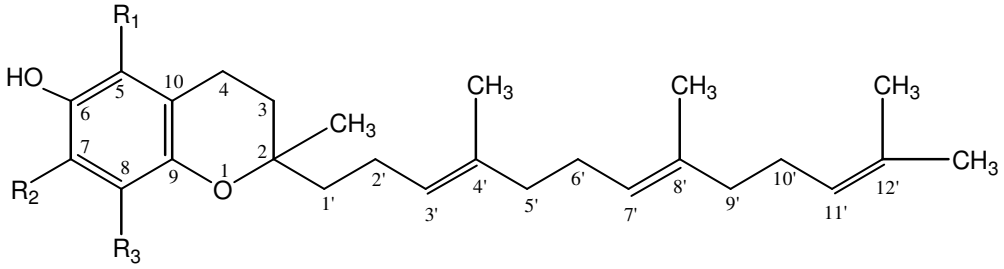
Vitamin E is the term suggested for fat-soluble 6-hydroxychroman compounds (tocol and tocotrienols derivatives) that exhibit biological activity of α-tocopherol by the rat resorption-gestation assay (Chow, 2001; Eitenmiller and Lee, 2004). The term "tocopherols" is the generic descriptor for all mono-, di- and tri-methyltocols and tocotrienols, and it is not synonymous with the term "Vitamin E" (Chow, 2001). The term "tocol" are trivial designation for 2-methyl-2 (4', 8', 12')-trimethyltridecychroman -6-ol (Stone and Papas, 1997).

Tocopherols and tocotrienols have a structure with a chromanol head and phytyl tail (side chain) (Rupérez *et al*., 2001). Tocopherols have a saturated side chain whereas tocotrienols have an unsaturated side chain with double bonds at position 3', 7' and 11'. They are characterized as α-, β-, γ, δ-tocopherol and α-, β-, γ, δ-tocotrienols by the degree and position of methylation in the 6-chromanol ring. (Figure 7).

Vitamin E is naturally synthesized only by plants (Tappel, 1992) and occur naturally as free alcohols. The synthesized ester form, acetate and succinate, has higher stability against oxidation. Synthetic water-soluble vitamin E is also available (Bender, 2003). Synthetic forms of vitamin E are used in food fortification and in vitamin supplements. Vitamin E supplements are sold as esters of either natural *RRR*- or the synthetic mixture (*all-rac*) forms of α-tocopherol (IOM, 2000). However, ester forms of vitamin E do not have antioxidant activity.



Tocopherol



Tocotrienol

Tocopherol and Tocotrienols	R_1	R_2	R_3
α-5, 7, 8-Trimethyl	CH_3	CH_3	CH_3
β-5, 8-Dimethyl	CH_3	H	CH_3
γ-7, 8-Dimethyl	H	CH_3	CH_3
δ-8-Methyl	H	H	CH_3

Figure 7 Structure of tocopherols and tocotrienols

2. Antioxidant Function of Vitamin E

Vitamin E is classified as a primary or chain breaking antioxidant that is effective in food and biological systems (Machlin, 1984; Burton *et al*., 1983). Antioxidant activity of vitamin E compound is due to the ability to donate phenolic hydrogen from chroman ring to lipid free radical (Pokorny, 1987; Burton and Ingold, 1989; Kamal-Eldin and Appelqvist, 1996). It takes the free radical out of the reaction to delays the initial step or interrupting the propagation step of autooxidation by donating a hydrogen to the acyl radical (R·, RO·, ROO·) resulting in more stable, non-radical products (RH, ROH, ROOH). This is because the antioxidant reacts faster to proteins or fatty acids side chains than acyl radicals (Burton and Ingold, 1989; Hamilton *et al*., 1997). The antioxidant radicals (A·) that are produced at this stage are more stable and less readily available to promote further oxidation (Eitenmiller and Lee, 2004; Fennema, 1996). The reduction of lipid free radicals delays the potential deterioration of highly reactive oxidizing species (Tappel, 1992).

Lipid oxidation is the most limiting factor in maintaining food quality, especially, when the use of polyunsaturated fatty acid from plant or fish oils in human nutrition is increasing (Wagner *et al*., 2001). The more unsaturated fatty acid in food, the higher requirement of vitamin E intake (Horwitt, 2001).

The relative effectiveness of tocopherols as antioxidants depends mainly on the chroman structure (Stone and Papas, 1997). The chemical structures of tocopherols and tocotrienols support a hydrogen donating power is in the order of $\alpha > \beta > \gamma > \delta$ (Pokorny, 1987). Giese (1996) found that γ- and δ-T3 had higher antioxidant activity that of γ-T and δ-T. The optimum concentration of tocopherols and tocotrienols as an antioxidant depend on many factors. Prooxidant activity may appear when the concentration is above concentrations that provide the maximum antioxidant activity, especially when the prooxidant synergists; transition metal, hydroperoxides, various oxidative oxygen species, heme proteins and photosynthesizers are in the system (Kamal-Eldin and Appelqvist (1996). Eitenmiller and Lee (2004) stated that the effectiveness of tocopherols and tocotrienols as

antioxidants is influenced by conditions influenced by experimental design, chemical properties of food matrix, and environmental factors that interact to increase the complexity of oxidation events.

Various forms of vitamin E are classified as generally recognized as safe (GRAS) when used in accordance with good manufacturing practices. Code of Federal Regulations (CFR) by United States Food and Drug Administration (USDA) limits the maximum levels in use of tocopherols in fat, oil, pork, meat, and poultry products to 0.03% based on fat content when there is no combination of other antioxidants (CFR 424.21). However, the level in use of tocopherols as a chemical preservative is GRAS (CFR 182.3890) (Office of the Federal Register, 2003).

3. Effect of Extrusion on Vitamin E

The severe conditions of temperature, pressure and intense mechanical shear active during extrusion affect physical and chemical properties of the extrudate. Changes in chemical composition are generated by thermal degradation, depolymerization and recombination of fragments. With these changes, vitamin E loss through oxidation can be quite pronounced. Destruction of natural antioxidants during extrusion is a major factor responsible for the susceptibility of extruded material to lipid oxidation.

Increased extrusion temperature reduced the retention of vitamin E in extruded rice bran at extrusion temperatures above 120°C (Shin *et al*., 1997). About two thirds (66.3-68.5%) of vitamin E in bread fortified with dl-alpha-tocopheryl acetate was retained after processing. No further significant change of vitamin E content was found during the seven-day-shelf-life of the product (Ranhotra *et al*., 2000).

Extensive degradation of vitamin E was found after drum-drying of whole-meal wheat-flour. Wheat flours were mixed, scalded and fermented and then drum-drying using process that common used to the manufacture of infant products. About 58% of alpha-tocopherol was degraded after mild drum-drying condition. The

authors concluded that the degradation of vitamin E was related to its function as an antioxidant (Wennermark *et al*., 1994).

MATERIALS AND METHODS

Materials

1. Fish Powder

Dry fish powder produced from Lizard fish (*Saurida* sp.) was purchased from P.N. Marine Food Products Co., Ltd., Samutsakorn, Thailand. The dry fish powder was packed under vacuum in plastic bags and stored in cardboard boxes at -20 °C until shipped. Each bag contained approximately 2 kg of fish powder. The fish powder was shipped to the United States by airfreight at ambient temperature. The shipping process required 7 days for arrival at the Department of Food Science and Technology, University of Georgia, Athens, GA, 30602. The fish powder was immediately stored at -20 °C after received. The composition of the fish powder according to the technical data sheet is shown in Table 3.

The fish powder was produced for human consumption. Total plate count was 8.2×10^2 cfu/g and *Escherichia coli* was less than 3 MPN. Salmonellae, *Staphylococcus aureus* and *Vibrio parahaemolyticus* were not detected.

Moisture, fat, eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA) contents were analyzed again before use.

2. Fish Oil

Refined menhaden oil was provided by Omega Protein, Inc., Refined Oil Division, Reedville, VA, U.S.A. The refined oil was reported to contain approximately 17% eicosapentaenoic acid (EPA) and 12% docosahexaenoic acid (DHA) (maximum level by weight) according to company specifications.

Table 3 Fish powder composition [a]

Components	Contents per 100g
Calcium (g)	2.35
Sodium (g)	1.32
Potassium (g)	0.31
Iodine (μg)	97.55
Eicosapentaenoic acid (EPA) (mg)	5.30
Docosahexaenoic acid (DHA) (mg)	19.90
Vitamin A (IU)	185.37
Vitamin D	ND
Fat (g)	3.39
Protein (g)	70.80
Carbohydrate (g)	0.05
Fiber (g)	0.48
Ash (g)	13.78
Moisture (g)	11.50
Calories (kcal)	313.91

[a] provided by P.N. Marine Food Products

3. Rice Flour

Medium grain rice flour (Rivland ™, RM-100) was purchased from Riceland Foods, Inc., Stuttgart, AK, U.S.A.

4. Mixed Tocopherols Concentrate

A mixed tocopherol concentrate (COVI-OX T70) containing approximately 70% by weight tocopherols was obtained from Cognis Corp., Cincinnati, OH, U.S.A. The composition, determined by liquid chromatography (LC), was 7041 mg/100g α-tocopherol, 510 mg/100g β-tocopherol, 34387 mg/100g γ-tocopherol and 19375 mg/100g δ-tocopherol.

Methods

1. Consumer Survey Conducted in Thailand

The consumer survey was conducted in 3 shopping malls located in Bangkok, Thailand during October 16-23, 2001. The consumers were asked for their willingness to participate; then the instruction and questions were explained to the participants. The questionnaire (Appendix A) was mainly composed of 3 parts; demographic information, snack eating and buying information, perception and attitude toward foods and snacks containing fish and/or fish oil. Data collected from three survey locations were combined, and unusable questionnaires were discarded. The frequencies of coded responses were analyzed using Statistical Package for the Social Science (SPSS®) version 12.0.

2. Experimental Design and Statistical Analysis

To achieve the objective of developing good products with optimal quality, it is very important to first identify the properties and levels that are important to a specific food quality parameter. A critical issue is the determination of the optimum

recipe and processing conditions to produce a food of high quality and marketability. Optimization is a procedure used for developing the best possible product in its class and in sensory evaluation, this means measuring the opinion or response for the most liked/preferred product (Sidel and Stone, 1983). There are many tools to be used in product development. Among the statistical experimental methods available, response surface methodology (RSM) is an effective method for food product development (Hu, 1999). Response surface methodology is a useful statistical and mathematical technique for developing, improving and optimizing processes (Mayer and Montgomery, 1995). The original work in this area dates from 1950s and has been widely used (Mayer *et al*, 2004). Use of RSM reduces the number of the treatments in the experiment necessary to provide sufficient treatment for statistically reliable results (Floros and Chinnan, 1987).

A 3×3×3 fractional factorial design suggested by Box and Behnken (1960) was used for RSM studies to determine the effects of barrel temperature, screw speed and feed moisture content on n-3 fatty acid and vitamin E contents and also on the physical and sensory properties of extrudates of the rice flour, fish powder and fish oil formulation during extrusion. Box-Behnken designs are mainly used to examine second order polynomials. The design is suitable for studies employing RSM (De Baun, 1959). A rotatable design for RSM requires at least five levels of each independent variable to be explored (Box and Behnken, 1960). Therefore, the total number of observations was fifteen, including three center points (0, 0, 0) and twelve forming a cuboctahedron (±1, ±1, 0; ±1, 0, ±1 and 0, ±1, ±1). The lack of experimental points at the vertices of the cubic region (±1, ±1, ±1) created by the upper and lower limits of each variable in this design is an advantage as these conditions are sometimes impossible to test due to physical process constraints (Montgomery, 1984).

A mixture consisting of 25% fish powder, 73.2% rice flour and 1.8% menhaden oil was formulated to provide approximately 300mg n-3 fatty acids/ 28g (dry basis). A mixed tocopherol preparation was added at 0.05% of the fat content as an antioxidant. The formulation was extruded with an APV Baker twin-screw

extruder (25:1 L/D) at 125-145°C, 150-300 rpm screw speed and at 19-23% moisture according to Box-Benhken experimental design (Table 4). Response surface methodology was used to determine the effects of barrel temperature, screw speed and feed moisture content on n-3 fatty acid and vitamin E contents and also on the physical and sensory properties of extrudates. Second order polynomial equations (Eq. 1) were used to generate the predictive regression model of the extrudate properties as functions of process variables.

$$Y = \beta_0 + \Sigma\, \beta_i X_i + \beta_{ii} X_i^2 + \Sigma\, \beta_{ij} X_i X_j \qquad \text{Eq. 1}$$

where: Y represents the experimental response, β_0, β_i, β_{ii} and β_{ij} are constants and regression coefficients of the model, and X_i and X_j are independent variables in coded values. The model includes linear, quadratic and cross product terms (Eq. 2).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 \cdot X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} \cdot X_3^2 \qquad \text{Eq. 2}$$

The coefficients of predictive regression model (Eq 2) were obtained by using RSREG in PROC GLM mode of Statistical Analysis System (SAS) (SAS Institute, 1996). Adequacy of the models was determined by R^2 and model lack of fit significant test ($p \leq 0.05$). The R^2 explains the proportion of variability that can be explained by data.

Model lack of fit is the measurement of the failure of a model to represent data in the experiment at which points were not included in the regression (Montgomery, 1984).

Response surfaces were illustrated by contour plots that represented the response in function of 2-factors when another is constant. Contour plots were generated from the models using STATISTICA software version 6 (Statsoft, Tulsa, OK). The optimum processing conditions were then determined by using RSM.

Table 4 Box-Behnken experimental design for extrusion of a fish powder, rice, fish oil formulation

Run	Coded independent variables			Uncoded independent variables		
	X_1	X_2	X_3	Temperature (°C)	Screw speed (rpm)	Moisture (%)
1	-1	0	1	125	225	23
2	-1	-1	0	125	150	21
3	-1	1	0	125	300	21
4	-1	0	-1	125	225	19
5	0	-1	1	135	150	23
6[a]	**0**	**0**	**0**	**135**	**225**	**21**
7	**0**	**0**	**0**	**135**	**225**	**21**
8	**0**	**0**	**0**	**135**	**225**	**21**
9	0	1	1	135	300	23
10	0	-1	-1	135	150	19
11	0	1	-1	135	300	19
12	1	0	1	145	225	23
13	1	-1	0	145	150	21
14	1	1	0	145	300	21
15	1	0	-1	145	225	19

[a] Values in bold represent the repeat center points (0, 0, 0)

The extrudates from 15 experimental conditions and raw material were analyzed for chemical, physical and sensory properties. The extrusion conditions that provided high retention of EPA, DHA with acceptable sensory quality were selected as an optimum region.

3. Raw Material Formulation and Mixing

3.1 Raw Material Formulation

The formulation was calculated on dry weight basis (db). Moisture, fat and vitamin E contents of the ingredients were analyzed (Table 5). The batch size per run was planned prior to the formulation calculation. Expected n-3 fatty acids from fish powder and added fish oil was calculated to be 900 mg/100g of the formulation. The fish powder was set to 25 g/100g of formulation (db). The fat content, EPA and DHA from fish powder was calculated based on analytical data (see 3.2). After that, added fish oil content was calculated to make the EPA and DHA contents to the desired level. Tocopherol content at 500 ppm was calculated based on the fat content from fish powder and fish oil. Rice flour content was calculated to complete the formulation. The formulation per 100 g mixture is given in Table 6. The actual amounts of each ingredient in the formulation were calculated according to Eq. (1).

$$\frac{\text{ingredient in formulation (g/100g)} \times \text{batch size}}{100 - \text{moisture of ingredient (g/100g)}} \qquad \text{Eq. 3}$$

3.2 Raw Material Mixing

All the ingredients were taken from the freezer and equilibrated to room temperature before mixing. All ingredients were weighed according to the formulation. The tocopherol concentrate was weighed into a 100 mL beaker and the exact weight was recorded. The required amount of fish oil was added into the tocopherol mixture. The fish oil and tocopherol concentrate premix was thoroughly mixed with a Teflon spatula and transferred into the fish oil container (3000 mL

Table 5 Moisture, fat, EPA, DHA and tocopherol contents of the ingredients [a]

	Fish powder	Fish oil	Rice flour	Tocopherols
Moisture (g/100g)	4.1	-	12.4	-
Fat (g/100g)	9.5	100	-	-
EPA (mg/100g)	11.0	6.3	-	-
DHA (mg/100g)	11.2	15.6	-	-
Mixed tocopherols (mg/100g)	0.94	108.1	0.49	61300

[a] mean of three determinations

Table 6 Extrusion formulation

Material	Amount in 100 g (db) (g)
Fish powder	25.0
Fish oil	1.78
Rice flour	73.2
Mixed tocopherols	0.001963
Water	Adjusted to the desired content during extrusion

beaker). About 5 kg of the fish powder and rice flour mixture were put into a stainless steel bowl, and the mixed fish oil-tocopherol preparation was added with mixing. The bulk of the fish powder and rice flour was mixed with a ribbon mixer (Model HD 1½ - 3SS, Munson Machinery Co., Utica, NY, U.S.A.) for about 5 min, then the fish oil-tocopherol mixture was added to the ribbon mixer. All ingredients were mixed for 30 min. The formulation was stored in three ply kitchen bags (GLAD®, Drawstring bag, The Glad Products Company, Oakland, CA, U.S.A.) at 10°C overnight to equilibrate the moisture. Three 200 g portions of the mixture from 3 positions in the mixer were removed and stored in linear low-density polyethylene (LLDPE) bags under vacuum at -50°C for compositional analysis at a later time. Calculated composition of raw material based on analysis of ingredients is shown in Table 7.

Since the n-3 fatty acids are susceptible to oxygen, we originally expected a significant loss of EPA and DHA during mixing. An airtight custom made plastic mixing drum was used to mix the ingredients. After loading the ingredients, the mixing drum was flushed with excess CO_2, then the drum was rolled by a drum roller for 30 min. The drums were immediately stored at 10°C overnight and brought to room temperature the next day before extrusion. However, initial studies showed that the retention of the fatty acids and vitamin E in the mixture after mixing in the airtight custom drum was not significantly different from the mixture that was mixed in the ribbon mixer (as described previously). Therefore, the ribbon mixer was selected to mix the ingredients due to ease of use.

4. Extrusion

4.1 Extruder

A co-rotating twin-screw extruder (Model MPF 1700-30, APV Baker Ltd., Newcastle-U-Lyne, England) with a length-to-diameter ratio of 25:1 was used for the entire study. A 3 mm opening tapered cylindrical die was used. The screw configuration consisted of four mixing zones, with forwarding and reversing paddles,

Table 7 Calculated composition of raw material based on analysis of ingredients

Raw material	Amount in 100g mixture	EPA (mg)	DHA (mg)	EPA+DHA (mg)	Tocopherols and tocotrienols (mg)
Fish Powder (g)	23.4	246.4	249.0	495.4	0.22
Fish oil (g)	1.6	103.2	249.6	352.8	1.73
Rice flour (g)	75.0	-	-	-	0.33
Vitamin E (mg)	1.965	-	-	-	1.66
Amount in 100 g					
Wet weight		349.5	498.6	848.2	3.94
Dry basis [a]		381.3	544.0	925.3	4.30

[a] Raw material mixture contains 8.4% moisture

interpersed with feed screw zones 1 (Figure 8). The extruder was equipped with an electrically heated clam-shell barrel with 4 temperature controlled zones and one non-temperature controlled zone before the die. Cooling water was circulated through the extruder barrel to maintain the temperature. The barrel at the feed hopper was not heated and was maintained at 25 to 30°C by cooling water. Dough temperature was measured by thermocouples that contacted the moving material at 3 locations in the barrel.

Pressure at the die and torque were recorded 3 times during the extrusion. The first record was taken when the extrusion conditions reached the desired temperature, stabilized pressure and torque. The second and third recording were at the middle and end of extrusion.

4.2 Sample Preparation for Extrusion

The formulation mixture was brought out from cold storage and equilibrated to room temperature before the containers were opened. The mixture feed rate (db) and water feed rate (mL/min) were calculated based on the calibration curve prepared before extrusion (Figure 9-A and B). To calibrate the material feed rate, the mixture with known moisture was filled to 2/3 of the volume of the feed hopper. The weights of the mixture from the feed hopper were recorded at 100, 200, 300, 400 and 500 rpm feed rates. A curve was produced to show the relationship between feed rates and the material output, where the Y-axis represented the material weights and the X-axis represented the feed rates (Figure 9-A). The desired feed rate (10 kg/hr, db) was calculated from the regression equation of the curve. The level of the ingredient mixture was maintained at 2/3 of the volume of the feed hopper during extrusion.

The moisture contents of the mixture were adjusted by adding the water directly to the extruder in the feeding zone during extrusion. A flow meter (Gilmont Accural ® Model GF-6541-1220, Gilmont Instrument, Berlington, IL, U.S.A.) was used to control the desired water delivery rate. Deionized water was used for all

experiments. Water delivery amounts were calculated by subtracting the moisture content from the raw material from the desired moisture level. The flow meter was connected to a 3.0 gal water reservoir pressurized (20 psi) with N_2. The water outlet from the flow meter was fed into the extruder in the feeding zone (Figure 9). Flow rates from the water system were calibrated by recording the weights of the water from the flow meter (30-90 mL/min) for every 5 mL/min incremental setting. A curve was plotted to show the relationship between the flow meter setting and the output of water (g), where the Y-axis represented the water weights, the X-axis represented the flow meter setting (mL/min) (Figure 9-B). The desired water feed rates were calculated based on the regression equation of the curve.

4.3 Extrusion Parameters

A modified screw configuration suggested by the manufacturer with feed screw, forward and reverse paddles was set up for all experimental runs. The details of screw configuration No.6 are shown in Table 8. Materials were fed into the feeding zone inlet with a K-Tron™ volumetric feeder (Model K2VT20, K-Tron Corp., Pitman, NJ, U.S.A.). Extrusion parameters are given in Table 9 and as described in Table 4. The barrel temerature (zone, 2, 3 and 4) were set to the desired temperature as indicated by controllers on the extruder. The barrel temperature adjacent to the feeding zone and zone 1 were set at 100°C.

4.4 Sample Collection

When the extruder reached steady state, as indicated by constant values for extruder motor torque, pressure at the die, and dough temperatures, samples were collected for residence time determination (RTDs). After that, samples were collected for the chemical and physical analysis and for sensory evaluation. The collected extrudates were dried at 80° C for 10 min in an impingement oven. After the samples cooled down to room temperature, samples for chemical analysis were vacuum packed in polyethylene laminated alluminium foil bags. The remainder of the

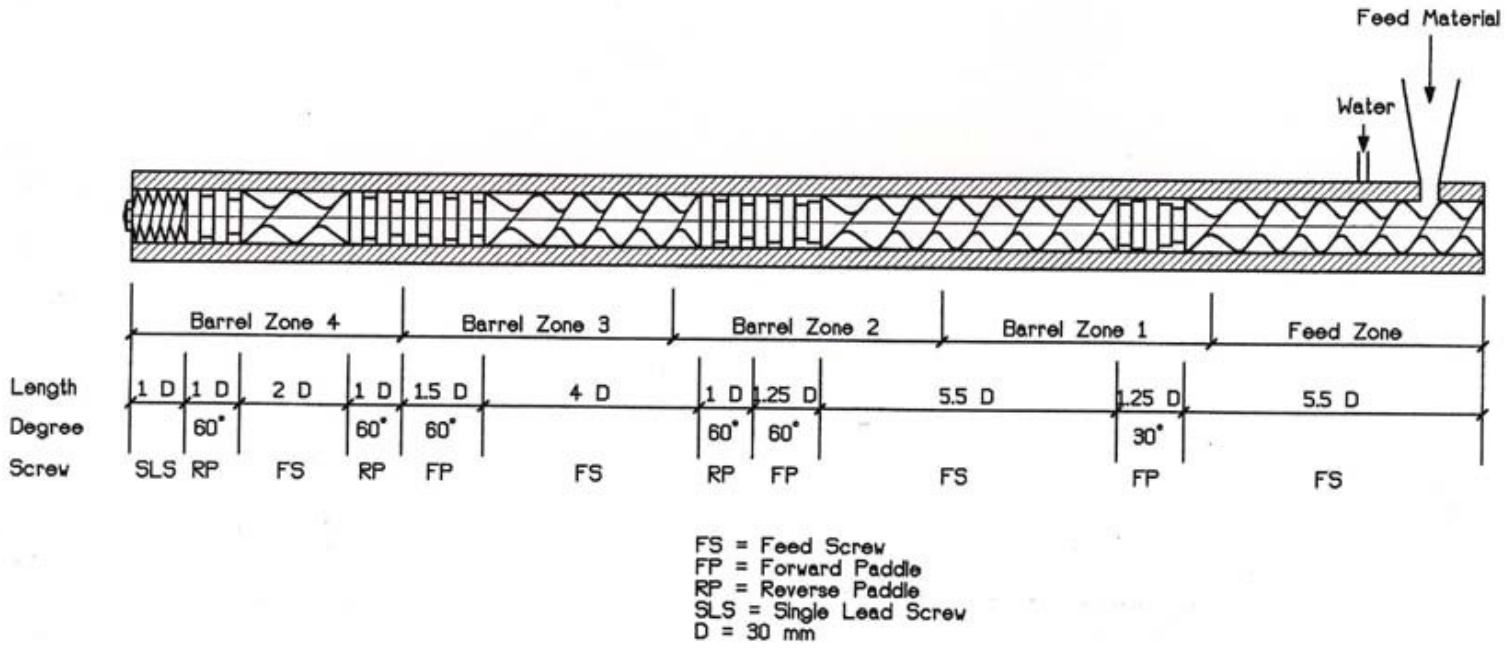
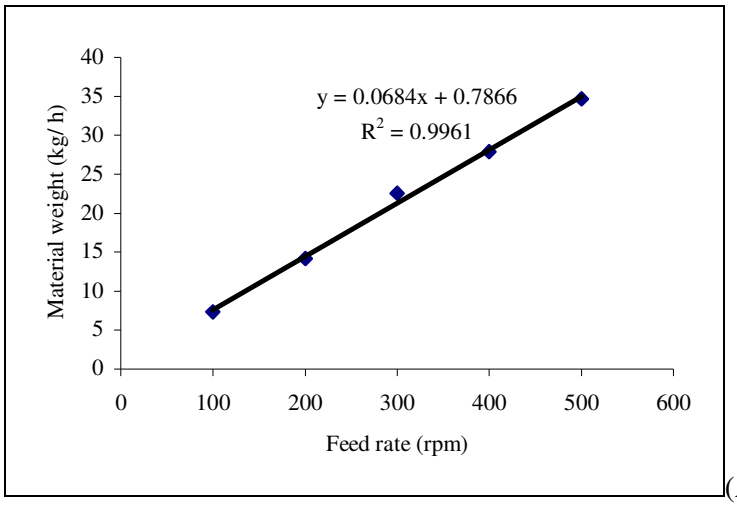
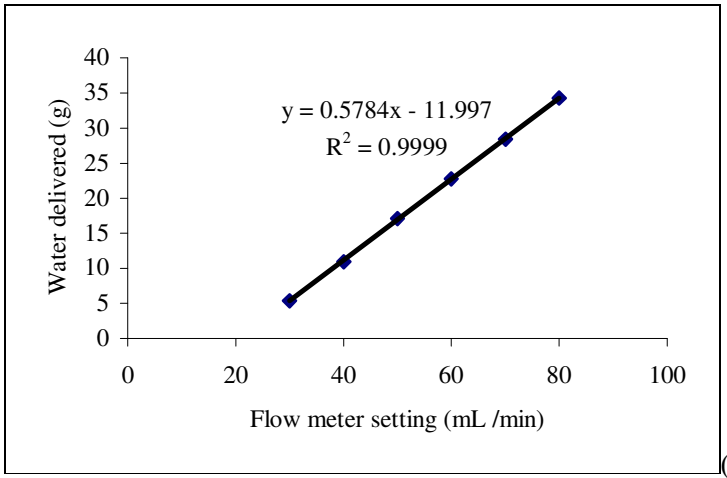


Figure 8 A side-view diagram of the screw configuration



(A)



(B)

Figure 9 Correlation between feeder feed rate (rpm) and material output (kg) (A) and water flow meter setting (mL/min) and water delivered (g) (B)

Table 8 Screw configuration [a]

Screw descriptions	Screw number	Screw length (D)[b]	Screw distance (D)	Cumulative distance (D)	Screw length (mm)	Screw distance (mm)	Cumulative distance (mm)
Feed screws	3	1.5	4.5	4.5	45	135	135
Feed screws	1	1.0	1.0	5.5	30	30	165
30° forward paddles	5	0.25	1.25	6.75	7.5	37.5	202.5
Feed screws	3	1.5	4.5	11.25	45	135	337.5
Feed screws	1	1.0	1.0	12.25	30	30	367.5
60° forward paddles	5	0.25	1.25	13.5	7.5	37.5	405
60° reverse paddles	4	0.25	1.0	14.5	7.5	30	435
Feed screws	2	1.5	3.0	17.5	45	90	525
Feed screws	1	1.0	1.0	18.75	30	30	555
60° forward paddles	6	0.25	1.5	20.0	7.5	45	600
60° reverse paddles	4	0.25	1.0	21.0	7.5	30	630
Feed screws	2	1.0	2.0	23.0	30	60	690
60° reverse paddles	4	0.25	1.0	24.0	7.5	30	720
Single lead screw	1	1.0	1.0	25.0	30	30	750

[a] The screw configuration was modified from APV Baker suggested screw configuration for oily feed.

[b] 1 D = 30mm

Table 9 Extrusion parameters

Parameter (Independent variables)	Range
Barrel temperature [a] (°C)	125-145
Screw speed (rpm)	150-300
Feed moisture (g/100g)	19-23

[a] Barrel temperature; zone 2, 3 and 4 located from the feeding zone

samples were stored in 1 gal Ziplock® bags for physical analysis and sensory evaluation. All samples were temporarily kept at 10° C. At the end of the day, the samples for chemical analysis were stored at -50°C, and the rest of the samples were stored at
-20° C.

5. Determination of Residence Time Distributions (RTDs)

5.1 Sample Collection

One milliliter of red food dye (FD&C Red #40, Warner Jenkinson Company, Inc., St. Louis, MO) was mixed with 5 g of raw material. The dyed raw material was fed into the feed inlet when the extruder conditions were in steady state. At the same time, the dyed raw material was added, a timer was started and the extrudate was collected every 10 s until the color visually disappeared (Yeh *et al*., 1992). All samples were separately kept in labeled Ziplock® bags.

5.2 Sample Preparation for Color Measurement

The samples were dried at 65-70°C for 8 h in a hot air oven (Isothem Oven, Fisher Scientific, Pittsburg, PA, U.S.A.). Fifty grams of dried sample were ground with a coffee grinder (Mr. Coffee, Cleveland, OH, U.S.A.) for 1 min. The ground powder was packed in clear 35x10 mm polystyrene cell culture dishes (CORNING®, Corning Corp., NY, U.S.A.). A Chromameter (Minolta CR-300, Minolta Corp., Ramsey, NJ, U.S.A.) was used to measure and calculate color of the samples based on 2° standard observer D65 illuminant. Three measurements per sample were made for the CIE L*, a*, b* values. Means of the redness (a*) were used in the calculations of the RTD values.

5.3 Data Analysis for RTDs

The mean residence time (t_m) is the time that the material spends in the extruder (Yeh *et al*., 1992). Mean residence time is calculated from the residence time distributions of the material in the extruder that are usually described as E(t) and F(t) functions and their curves (Peng *et al*., 1994). E(t) is the exit age (differential) distribution function and F(t) is the cumulative distribution function (Unlu *et al*., 2002). The E curve shows the exit age distribution and is plotted as normalized concentration E(t) vs. residence time (t). The F curve is the plot of cumulative E(t); F(t) vs. normalized time (residence time (t)/ mean residence time(t_m)) (Levenspiel, 1972, Lee and McCarthy, 1996).

E(t) and F(t) functions are represented by following formula:

$$E(t) = \frac{c}{\int_0^\infty c\,dt} \approx \frac{c_i}{\sum_{i=0}^{\infty} c_i \Delta t}$$

where c is the tracer (here is the redness; a*) concentration at time t

$$F(t) = \int_0^t E(t)dt \cong \frac{\sum_{i=0}^{i=t} c_i \Delta t}{\sum_{i=0}^{i=\infty} c_i \Delta t}$$

The mean residence time (t_m) is given by

$$t_m = \int_0^t tE(t)dt \cong \frac{\sum_{i=0}^{\infty} t_i c_i \Delta t}{\sum_{i=0}^{\infty} c_i \Delta t}$$

Microsoft Excel was used to calculate and plot E(t) and F(t) functions.

6. Chemical Analysis

6.1 Moisture Determination

About 3 g of ground sample was weighed into an aluminum pan. The exact weight of the weighing pan and the sample were recorded before and after drying in the vacuum oven at 70±5 °C and 22-25mmHg for 6 h. Moisture content determinations were done in 3 replicates. The moisture content was calculated by the following equation (Eq. 4):

$$\text{Moisture content (g/100g db)} = 100 - \frac{(\text{Sample wt. before dried} - \text{sample wt. after dried}) \times 100}{\text{Sample wt}} \quad \text{Eq. 4}$$

6.2 Fatty Acid Analysis

6.2.1 Lipid Extraction

Addition of fatty acids changes the physical and chemical properties of starchy foods (Singh *et al*., 1998). In cereals, substantial amounts of lipids are bound to gelatinized starch and other complex carbohydrates, making lipid extraction difficult. Use of conventional fat extraction procedures based on acid hydrolysis, hot water-butanol, ether, hexane, combination of non-polar solvents and long Soxhlet extraction can damage the lipids, making the extract unsuitable for fatty acid analysis. This becomes more problematic with highly unsaturated fatty acid such as EPA and DHA that are inherently unstable. Additionally, traditional fat extraction procedures are not capable to completely extract lipid from cereals, and, more importantly to this study, from extruded cereal products (Kaur and Singh, 2000; Strange and Schaich, 2000). First attempts in this study to extract the lipid from the extruded products with hexane using Soxhlet technique dramatically showed that the procedure was incapable of efficiently extracting the lipid. Extracted lipid only reached 50-60% of the predicted lipid available from the ingredients. Extrusion and other cooking processes used for cereal products have been shown to encapsulate lipids in a protein-starch

matrix (Davis, *et al.*, 1986; Fanta and Eskins, 1995; Strange and Schaich, 2000; Kaur and Singh, 2000).

To counteract the lipid extraction problem with extruded products, Strange and Schaich (2000) developed an extraction procedure based on α-amylase digestion followed by 2:1 (v/v) dichloromethane: methanol or 2:1 (v/v) chloroform: methanol extraction. The procedure extracted > 97% of the total lipid from extruded corn-soy products. It was, therefore, decided to use their method in this research. The procedure included the following steps:

1.) Sample preparation: Samples were allowed toequilibrate to room temperature. Approximately, 35-40 g of sample were ground for 1 min. Three replicates of each sample were extracted. All phases of the extraction were completed under gold fluorescent lighting or in darkness.

2.) α-Amylase digestion: Ten grams of ground sample were placed into a 300 mL Erlenmeyer flask. Fifty milliliters of deionized water containing 250 mg α-amylase (α-amylase type VIII-A from Barley Malt [EC 3.2.1.1] containing 2.0 units α-amylase/mg solid and 3.0 units β-amylase/mg solid, Sigma Chemical Co., Ltd., St. Louis, MO, U.S.A.) was then added. The sample was flushed with nitrogen gas, capped with ground glass cap and sealed with paraffin film, and incubated at 37 °C for 16 h in a shaking water bath at 100 rpm.

3.) Fat extraction: After digestion, the sample digest was transferred into a 500 mL separatory funnel with 250 mL of 2:1 (v/v) chloroform: methanol. The funnel was inverted and shaken to partition the lipid into the organic phase. The phases were allowed to partition for 2 h. The lower partitioned phase containing the lipid was approximately 150 mL. This phase was transferred to a 500 mL round bottom flask by filtering through a 150 mL fritted glass funnel (PYREX No. 36060, ASTM 10-50) containing a layer of 30 g of magnesium sulfate on Whatman No. 1 filter paper to remove water from the extract. The filter cake and funnel were rinsed

with 100 mL of chloroform. The organic solvent phase was then evaporated to dryness with a vacuum rotary evaporator (Büchi Rotavapor R110, Brickman Instruments, Westboro, NY, U.S.A.) at 45 °C. The lipid was quantitatively rinsed from the round bottom flask with 8 mL of hexane into a 13 × 100 mm test tube of known weight. The hexane was evaporated under nitrogen and the weight of the oil was determined. The test tube was flushed with nitrogen, sealed with paraffin film and stored at –50 °C until the fatty acids were quantified.

6.2.2 Fatty Acid Methylation

Fatty acid profiles of the lipid fraction were determined by the method of Amer, *et al*. (1985). Two hundred milligrams of anhydrous oil were weighed into a 25 mL (13 × 130 mm) Teflon lined screw cap tube. The lipid was dissolved in 2.0 mL petroleum ether, and 0.1 mL of sodium methoxide (2 N sodium hydroxide in anhydrous methanol) was added. The methylation mixture was mixed for 1 min with a vortex mixer, flushed with nitrogen and kept at –20 °C to ensure the sedimentation of sodiumglycerorate. The clear supernatant was transferred to a 4-drams amber vial, flushed with nitrogen, capped and stored at –50 °C until analyzed for fatty acids. Before analysis, the extract of methylester was equilibrated to room temperature and diluted 1:100 with hexane.

6.2.3 Gas Chromatography

About 1 μL of the hexane solution was injected in to a HP-FFAP capillary column (25m × 0.2 mm ID × 0.3 μm film thickness) (Agilent Technologies, Palo Alto, CA, U.S.A.). The column was installed in a HP5980 Series II gas Chromatograph (Hewlett-Packard, San Fernando, CA, U.S.A.) equipped with a flame-ionization detector (FID) and on-column injector. The carrier gas was nitrogen gas at a flow rate of 20 mL/min with hydrogen and air being supplied to the FID at a flow rate of 33 and 400 mL/min, respectively. The oven temperature was programmed as follows: initially held at 40°C for 5 min, and then increased at a rate of 20°C/min until

a temperature of 220°C was reached. The oven was held at this temperature for 30 min. The injector and detector temperatures were 250°C and 270°C, respectively. The fatty acid profile was based on the peak area that was integrated by using computer software interface HP3365 Series II Chem Station. Fatty acid methyl ester (FAME) peaks were identified by comparing the retention times with those of a standard mixture of 0.5 mg/mL in hexane of EPA (cis-5, 7, 11, 14, 17-eicosapentaenoic acid methylester, Sigma St. Louis, MO, U.S.A.) and 0.5 mg/mL in hexane of DHA (cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid methylester, Sigma, St. Louis, MO, U.S.A.) The fatty acid contents were calculated from the following equation (Eq. 5):

$$\text{Fatty acid (mg/100g)} = \frac{(\text{Avg. peak area \% of fatty acid} \times \text{mg of fat/ 100g sample})}{\text{Sample weight (mg)}} \times 100/\text{solid fraction} \quad \text{Eq.5}$$

6.3 Liquid Chromatography (LC) Quantification of Tocopherols and Tocotrienols

6.3.1 Sample Preparation

Saponification was used to extract the tocopherols and tocotrienols. The extrudates were kept under vacuum in laminated aluminium foil bags and stored at –50°C until analyzed. The sample preparation method was modified from Ye *et al*. (2000). A 1.5 g sample aliquot was accurately weighed into a 4 × 16 cm glass saponification tube. Ten milliliters of 6% pyrogallol (PG) in ethanol (EtOH) was added to the sample. The tube was flushed with nitrogen gas for 1 min and sonicated at room temperature for 5 min. After which, 3 mL of freshly prepared 60% (w/v) sodium hydroxide (NaOH) was added. The tube was flushed with nitrogen gas for 1 min and immediately fitted with an air condenser. The mixture was refluxed in a shaking water bath (120 rpm) at 70°C for 30 min. The saponification mixture was cooled with ice water and sonicated for 5 min at room temperature.

For extraction of the saponification digest, 20 mL of 2% (w/v) sodium chloride (NaCl) was added to the digest and mixed. Twenty-five milliliters of extraction solvent (10% ethyl acetate in n-hexane, v/v) containing 0.01% butylated hydroxytoluene (BHT) was then added. The digest was shaken vigorously and allowed to stand until phase separation occurred. The hexane layer (upper phase) was removed and transfered to a 50 mL volumetric flask by filtering through a layer of magnesium sulfate on Whatman No.1 filter paper to remove water from the extraction solvent. The entire extraction was repeated one time. Contents of the volumetric were brought to volume with extraction solvent. Prior to injection, the diluted extract was filtered through a 0.45 μm nylon membrane filter (Micro Separation Inc.; MSI, Westboro, Mass., U.S.A.).

6.3.2 LC Analysis

Tocopherols were quantified by normal-phase high performance liquid chromatography (HPLC). The LC system consisted of a LC-6A pump equipped with an RF-10A spectrofluorometric detector (Shimadzu Corp., Columbia, Md., U.S.A), and a SpectraSeries AS100 autosampler (Thermo Separation Products Inc., California, U.S.A.), and a 25cm × 4mm, 5μm LiChrosorb Si60 column (Hibar Fertigsaule RT., Darmstadt, F.R. Germany) equipped with a pre-column packed with Perisorb A 30-40μm (Darmstadt, F.R. Germany). The isocratic mobile phase contained 0.8% isopropanol (LC grade, Fisher Scientific, Pittsburgh, PA, U.S.A.) in n-hexane (LC. Grade, J.T. Baker, Phillipsburg, NJ, U.S.A.). The flow rate was 1.0 mL/min. The mobile phase was filtered using a 0.2 μm nylon membrane filter (Micro Separation Inc.; MSI, Westboro, Mass., U.S.A.). The concentrations of tocopherols in the samples were calculated, using the average peak areas compared between standards and samples at 290 and 330nm for excitation and emission wavelength, respectively, after duplicate injections. Mixed tocopherol standard working solution was prepared as follows:

Tocopherol standard solutions preparation

1.) Tocopherol pre-solution (10mg/5mL). Individual tocopherols standard were obtained from Sigma (St. Louis, MO, U.S.A.). Twenty milligrams of α-tocopherol (D, L-α-tocopherol) was dissolved in n-hexane and transferred to a 10 mL volumetric flask. The volume was adjusted with n-hexane. The γ-tocopherol and δ-tocopherol standards were prepared similarly.

2.) Stock solution I (0.6 mg/mL). Eight milliliters of tocopherol pre-solution was pipetted to 25 mL volumetric flask. Volume was adjusted with 0.1 % (w/v) BHT in n-hexane. Stock solutions for other vitamin E forms were prepared in a similar manner.

3.) Standard working solution I preparation (2 mg/mL).
One milliliter of tocopherol pre solution was pipetted into a 25 mL volumetric flask and the volume was adjusted to the level with ethanol. Other standard working solutions were prepared similarly. The absorbance difference ($A-A^0$) for each standard working solution was determined with spectrophotometer at a suitable wavelength, using setting given in Table 10. A is absorbance of the standard solution and A^0 is absorbance of the blank (ethanol). Concentration of each standard working solution was calculated from $E^{1\%}_{cm}$ data.

4.) Standard intermediate solution (1.2 mg/100mL). Mixed standard solution containing α-, γ-, δ-tocopherol was prepared. Two milliliters of each stock solution I was pipetted into 100 mL volumetric flask and the volume was adjusted the scale with hexane-BHT solution.

5.) Standard working solution II (containing 14, 48 and 1200 ng tocopherol/mL). One milliliter of standard intermediate solution was pipetted into 10 mL volumetric flask and the volume was adjusted to the level with hexane-BHT solution to obtain “std 1” (1200 ng tocopherol/mL). Pipetted 1 mL of “std 1” into 25 mL flask and dilute to the volume with hexane to obtain “std 2” (48 ng tocopherol /mL). Three milliliters of “std 2” was pipetted into 5 mL volumetric flask, and the

Table 10 Specific absorption coefficients ($E^{1\%}_{cm}$) and maximum wavelength (λ_{max}) for tocopherols in 96% (v/v) ethanol solutions.

Vitamer	λ_{max} nm	$E^{1\%}_{cm}$
α-T	292	70.8
γ-T	298	92.8
δ-T	298	91.2

Source: Ball (1988)

volume was adjusted to the scale with hexane-BHT to obtain “std 3” (14 ng tocopherol/mL). Standard working solution II was prepared on the day of used.

7. Analysis of Physical Properties

7.1 Product Density and Expansion Ratio

Samples were placed on the trays and put in a forced air oven at 60°C for 8 h. Samples were taken out from the oven and kept in Ziplock® bags at room temperature until analyzed.

The dimensions and weights of 10 strands of extrudate from each treatment were taken using a digital Vernier caliper (Sylvia digital Vernier calipers, Ultra-cal Mark III). The volume of each cylindrical extrudate was calculated by the average diameters and lengths of each extrudate. The product density (g/cm^3) was calculated by dividing each sample weight by its volume. The expansion ratio was calculated by dividing the cross section area of the extrudate with the cross section area of the opening die.

7.2 Shear Strength

A standard Kramer Shear-Compression cell was used to determine the shear strength. Dry strands of extrudate were placed to cover the bottom of the cell in a single layer. The maximum force required to break the sample was recorded with an Instron Universal Testing machine (Model 1122, Instron Corp., Canton, MA, U.S.A.) with a 50 kg load cell. The operating conditions were 50 mm/min cross-head speed and 50 mm/min chart speed. The maximum force to break the extrudates per cross-sectional area of extrudates was determined as shear strength (N/cm^2). The averages from five determinations per sample were taken.

7. Sensory Evaluation

Since the product is designed for the Thai market, all participants were Asian with no known food allergy. The participants were primarily from the university community. Thai students and their families were contacted through the Thai Student Association membership. Asian faculty in the Department of Food Science and Technology were asked to participate. The participants were asked to sign the consent form (Appendix B) and read the instructions (Appendix C). A set of five samples were randomly served and evaluated by each participant. The participants were asked to indicate their liking on the attributes (appearance, color, aroma, odor, flavor, texture and overall acceptability) of the samples on the hedonic scale of liking (Appendix D). The results were analyzed using the Statistical Analysis System (SAS®, SAS Institute, 1996).

RESULTS AND DISCUSSION

1. Consumer Survey Conducted in Thailand

Seven hundred consumers were recruited, however, only 652 questionnaires were usable. The frequencies of coded responses from 652 usable questionnaires were analyzed using SPSS® version 12.0. The frequencies of the responses are presented in percentages as shown in Tables 11-13.

1.1 Demographic Characteristics of the Participants

The demographic data are presented in Table 11. The participants were 31.9% male and 68.1% female, predominantly in the age of 15-35 years old (87.0%). Sixty-eight percent had high school and higher education. Fifty-five percent were students, 5.5% government employees, 19.5% company employees, and 4.4 % owned their business or were self-employed. Seventy-seven percent had individual incomes of less than 5,000 Baht to 10,000 Baht per month.

1.2 Consumer Responses to Question about Snack and Purchasing Decisions

The responses to questions about snacks and purchasing decisions are presented in Table 12. About 20% of the consumers regularly consumed snacks made from starchy material between meals. About 79% ate snacks everyday and 2-3 times a week. Fifty percent and 26.2% consumed snacks during free time and while watching television, respectively. About 51% consumed snacks in the afternoon (1:00PM-5:00PM). Most of the consumers (82.4%) spent more than 16-25 Bath for a snack. Most preferred snacks with strong and spicy favor (33.1%). Salty, original flavor of raw material, and sweet flavors were preferred by 23.0%, 19.3% and 14.3%, respectively. A very low density, highly puffed product was preferred by 49% of the participants.

Table 11 Demographic characteristics of the survey participants

Variable	Percentage
Gender	
Male	31.9
Female	68.1
Age (years)	
less than 15	4.4
15-20	37.3
21-25	32.4
26-35	17.3
35-45	7.2
Over 45	1.4
Education	
Primary school	2.1
Middle school	6.6
High school or equivalent	24.8
Certificate from 2 years college	8.3
4 Years college	51.7
Graduate school or higher	6.5
Occupation	
Housewife	4.6
Government employee	5.5
Company employee	19.5
Own business/ self employed	4.4
Labor	5.5
Agriculture	1.1
Student	55.5
Others, please specify…….	3.8

Table 11 (continued)

Variable	Percentage
Individual income per month (Baht)	
Less than 5,000	44.5
5,001-7,000	19.6
7,001-10,000	13.0
10,001-15,000	9.2
15,000-20,000	5.7
20,001-25,000	2.3
25,001-30,000	1.4
30,000 and higher	4.3

Table 12 Consumer responses to questions about snacks and purchasing decisions

Variable	Percentage
What kind of food you usually have between meals? (You can answer more than one.)	
Thai desert	10.6
Bakery products	29.6
Snack made from starchy material	19.8
Thai style between meals food	9.2
Potato chips	28.5
Fruits	46.2
Nuts and seeds	7.2
How often do you eat snacks?	
Everyday	34.0
2-3 times a week	44.7
Once a week	13.2
2 times a month	3.2
Once a month	2.1
Less than once a month	2.8
Main reasons for eating snacks (you can answer more than one)	
Between meals	13.7
Instead of meal	1.5
Eat while drinking	7.4
Eat when has free time	50.0
Eat while traveling	19.3
Eat while watching TV	26.2
Eat occasionally with different reason	39.6

Table 12 (continued)

Variable	Percentage
What time do you usually have snacks?	
Before 10:00AM	3.1
10:00AM-1:00PM	10.3
1:00PM-5:00PM	50.8
5:00PM-8:00PM	23.3
After 8:00PM	12.9
How much do you usually pay for a snack?	
5 Baht	1.8
6-10 Baht	8.0
11-15 Baht	7.8
16-20 Baht	22.1
21-25 Baht	13.7
More than 25 Baht	46.6
Where do you usually buy snacks?	
Convenience store	46.9
Local store	11.5
Supermarket	37.4
Wholesale store	1.8
Others	2.3
What flavor of the snack do you prefer?	
Original flavor of the raw material	19.6
Salty	23.0
Sweet	14.3
Strong flavor and spicy	33.1

Table 12 (continued)

Variables	Percentage
What kind of texture of the snack do you like?	
Puff, very low density	49.1
Puff, more dense	11.2
Lightly puff, dense	27.1
Dense, brittle	7.4
Dense and hard	5.3

1.3 Consumer Perception and Attitude toward Foods and Snacks Containing Fish or Fish oil.

Consumer perception and attitude toward foods and snacks containing fish or fish oil is presented in Table 13. The consumers thought that snacks had low (39.0%) to moderate (50.3%) nutritional value. Eighty-six percent liked foods that contained fish. About 86% had heard about beneficial health effects of fish oil. About 71% had heard the terms "EPA and DHA". The main source of their information about fish oil, and EPA and DHA was television and radio (62.7%). About 60% had purchased food, snacks or supplements containing EPA and DHA, and only 27.5% had purchased snacks containing fish oil. About 89% were willing to buy a snack containing fish oil.

Responses pertaining to perception and attitude showed that products containing fish and fish oil were acceptable by the consumer. Most consumers were aware of the beneficial heath effects of fish oil. Therefore, there is a potential to develop a nutritious snack that would be accepted by Thai consumers. Consumers responses were used to set guidelines for development of a snack product supplemented with n-3 fatty acids. The consumers preferred low density and highly puffed snacks with strong and spicy flavor. The demographic characteristics and the responses of consumers on the snacks and purchasing decisions are needed to develop target consumer groups, pricing, and marketing approaches.

Table 13 Response to questions about foods containing fish and/or fish oil

Variable	Percentage
What do you think about the nutritional value of the snacks available in the market?	
No nutritional value	4.0
Low nutritional value	39.0
Moderate nutritional value	50.3
High nutritional value	2.1
I have no idea	4.6
How do you like food that contains fish?	
Like extremely	18.1
Like very much	20.6
Like moderately	38.8
Like slightly	8.6
Neither like of dislike	8.0
Dislike slightly	1.4
Dislike moderately	2.5
Dislike very much	1.1
Dislike extremely	1.1
Have you ever heard about the heath beneficial effects of fish oil?	
Yes	86.3
No	13.7
Have you ever heard EPA and DHA?	
Yes	70.9
No	29.1

Table 13 (continued)

Variable	Percentage
What is the source of your information about health benefits of fish oil or EPA and DHA?	
TV or radio	62.7
Magazine	31.4
Scientific publication	31.1
Leaflet, brochure	8.3
Food exhibition	8.3
Friend or personal contact	9.8
Have you ever had food, snack or supplement that were labeled EPA and DHA?	
Yes	59.0
No	41.0
Have you ever had a snack containing fish oil?	
Yes	27.5
No	72.5
Are you willing to buy a snack that contains fish oil?	
Yes	88.6
No	13.2

2. Effects of Extrusion Conditions on EPA, DHA and Vitamin E Retentions, Processing Parameters, Physical and Sensory Properties of Extruded Fish Snacks Supplemented with n-3 Fatty Acids

Typically, extrusion studies examine only two or three primary extrusion variables, but many factors such as barrel temperature, die geometry, extruder type, feed composition, feed moisture, feed particle size, feed rate, screw configuration and screw speed can influence product quality. Secondary extrusion variables such as specific mechanical energy (SME), product temperature (PT) and pressure also influence the viscosity of dough within the barrel, the residence time of the material in the extruder and the shear applied to the food material. Chemical reactions that occur during extrusion have been extensively discussed (Meuser and van Lengerich, 1984 and Camire, 2000). Temperature, pressure and mechanical shear during extrusion affect physical and chemical changes occurring in the dough (Shin *et al*., 1997). Chemical changes primarily occur by thermal degradation, depolymerization and recombination of fragments produced from the dough ingredients (Camire, 2000).

Extrudates from the 15 experimental runs were analyzed for chemical, physical and sensory properties as described in the materials and methods section. The data obtained were analyzed for the effects of extrusion conditions (temperature, screw speed and feed moisture content) on dependent variables (EPA, DHA and vitamin E) as well as physical properties (product density, expansion ratio and shear strength) and sensory properties. The results were used to optimize the extrusion process variables that provide high stability of EPA and DHA and sensory acceptability. The process parameters were recorded during extrusion.

2.1 Effects of Extrusion Variables on Process Parameters.

Extruders combine several unit operations at the same time, during extrusion cooking. They convey and mix ingredients along the barrel, while exposing them to heat, pressure and shear forces. Ingredient compositions (starch, protein, sugar, salt, fat etc.) and extrusion conditions (feed moisture content, screw speed,

screw configuration, barrel temperature and die geometry) affect the final product quality through their influence on the extruder responses (motor torque, pressure, product temperature and shear). Motor torque, die pressure, barrel fill and, barrel temperature affect the specific mechanical energy input (SME) and residence time distribution (RTD) during extrusion cooking (Unlu and Faller, 2002). The SME relates the specific work input from the motor to the material being extruded and the material transformation leading to variation in expansion, density and geometric characteristics. Product temperature, mean residence time and SME have been used to predict physical properties of puffed extruded products such as expansion index, bulk density, as well as sensory characteristics (Meucer *et al*., 1986). Extrusion, being a high temperature, high pressure and short time process, has the advantage of retaining otherwise heat labile components such as vitamins in a food material. This study ultimately attempts to develop an extrusion process for an acceptable snack product with optimum content of n-3 fatty acids. This portion of the study was focused on the effects of extrusion variables on process parameters (dough temperature at the die, pressure at the die, motor torque, SME and RTD).

In all experimental runs, the extrusion process parameters (dough temperature at the die, pressure at the die and motor torque) were monitored by taking triplicate recordings of each parameter during the extrusion. The specific mechanical energy input (SME) was calculated from rated screw speed (500 rpm), motor power rating (6 hp), actual screw speed, % motor torque and the motor power rating as following formula (Eq. 6) (Gogoi *et al*., 1996b).

$$\text{SME} = \frac{\text{actual screw speed}}{\text{rated screw speed}} \times \frac{\%\ \text{torque}}{100} \times \frac{\text{motor power}}{\text{mass flow rate}} \qquad \text{Eq. 6}$$

The data obtained (Table 14) were analyzed by regressing the processing parameters (dough temperature at the die, pressure at the die, motor torque, SME, mean residence time) against the three extrusion variables (barrel temperature, screw speed and feed moisture content). Predictive regression models were developed and used to determine the influence of extrusion variables on each of the process parameters. The regression models for dough temperature at the die, pressure at the

die, motor torque (%), SME and RTD showed high R^2 of 0.97, 0.90, 0.97, 0.95 and 0.92, respectively. None of the models showed significant lack of fit (Table 15), suggesting that they are adequate and can be used to predict and explain the effects of extrusion variables on the processing parameters. However, in the case of product temperature and pressure at the die, none of the regression parameter coefficients showed significant effects to the model (Table 15).

2.1.1 Product Temperature

The recorded temperatures of the product at the die before exit were higher than the set temperature at the last zone of the extruder barrel. The model for product temperature at the die had R^2 of 0.97 and had no significant lack of fit (Table 15). The contour plots (Figure 10-A) show that increasing screw speed raised the product temperature at the die. Increasing feed moisture lowered the temperature at the die. Frame (1994) suggested that the heat during extrusion generated from interparticulate friction and friction amoung material, screw elements and barrel caused an increase in the die temperature.

2.1.2 Pressure at the Die

Pressure at the die in all the extrusion runs ranged from 2.7×10^6 to 4.7×10^6 Pa. The highest pressure at the die was observed at intermediate barrel temperature (135°), high screw speed (300 rpm) and low feed moisture (19%) (Table 14). The model showed a high R^2 of 0.90 and a non-significant ($p>0.05$) lack of fit (Table 15). Contour plots show that increasing feed moisture increased pressure at the die but reduced it at high feed moisture (Figure 10-B). Similar results had been reported by Pan *et al*. (1998) who observed that increasing moisture content of the feed significantly reduced die pressure in starch based materials. Increasing screw speed, at constant feed rate, was also observed by Lu *et al*. (1992) to reduce pressure at the die.

Table 14 Recorded values of processing parameters during extrusion

Experimental [a]				Product temperature [b] (°C)			Pressure at die [c]	Torque [d] (%)	SME [e] (kJ/kg)	t_m (s)
Run	Temp	SS	M	Tc_1	Tc_2	Tc_3	(Pa×10^6)			
1	125	225	23	120-121	120-125	124-126	3.1±0.07	23 ± 0.6	164±4.2	102.3
2	125	150	21	120-121	123-129	124-125	4.0±0.01	32 ± 1.5	156±7.4	105.6
3	125	300	21	117-118	122-128	125	4.0±0.48	22 ± 0.6	216±5.6	98.0
4	125	225	19	120-121	131	124-129	4.0±0.39	31 ± 0.5	221±3.6	95.1
5	135	150	23	117-118	135-136	136	3.3±0.17	25 ± 1.2	119±5.6	102.9
6 [f]	**135**	**225**	**21**	**119-120**	**131-135**	**134-136**	**3.2±0.09**	**25 ± 1.0**	**178±7.2**	**89.9**
7	**135**	**225**	**21**	**120-122**	**130-132**	**135**	**3.2±0.24**	**22 ± 0**	**160±0.0**	**93.9**
8	**135**	**225**	**21**	**118-120**	**130-134**	**134-137**	**3.7±0.12**	**24 ± 1.0**	**172±7.5**	**90.3**
9	135	300	23	122	131-132	134-136	2.2±0.14	17 ± 1.2	161±11.2	99.2
10	135	150	19	126	132-135	133-135	4.5±0.19	34 ± 0.6	163±2.8	111.3
11	135	300	19	118-119	130-135	143-147	4.7±0.19	24 ± 1.9	234±18.3	87.4
12	145	225	23	122-123	136-137	143-146	2.7±0.10	17 ± 1.5	126±11.1	96.9
13	145	150	21	124-125	140-148	146-149	3.5±0.10	26 ± 0.6	127±2.8	109.4
14	145	300	21	118-120	137-142	144-146	3.0±0.24	22 ± 1.3	208±12.8	83.4
15	145	225	19	119-122	135-143	145-147	3.9±0.78	23 ± 0.6	169±4.2	98.8

[a] Temp = temperature (°C) of barrel zone 2, 3 and 4, SS = screw speed and M = feed moisture (g/100g)

[b] Tc_1, Tc_2 and Tc_3 = dough temperature at zone 4: 12.5, 4.5 and 0 cm from die

[c, d] mean of three middle points from three readings

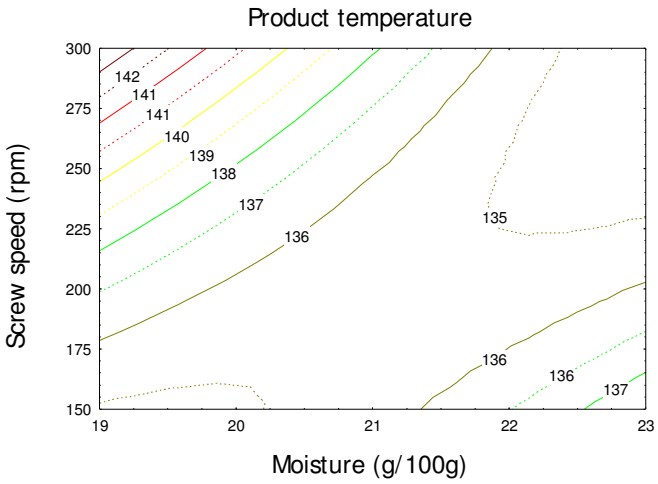
[e] SME = specific mechanical energy calculated from (% screw speed × % torque × motor power; kJ)/ feed rate; kg)

[f] the repeat center points of the experimental design

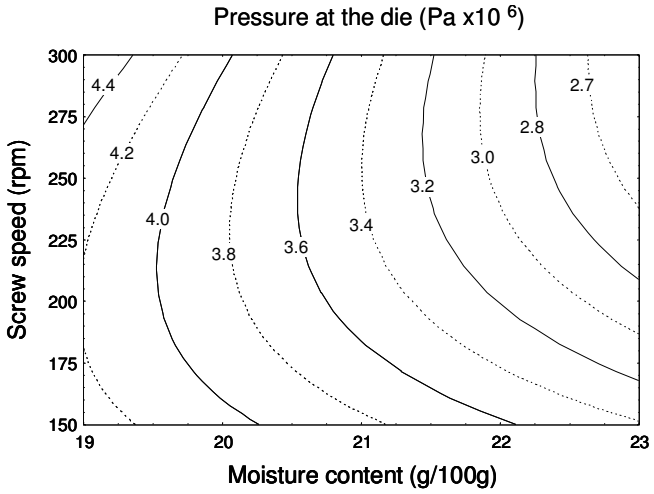
Table 15 Coefficients of parameters in the regression models for product temperature at the die, pressure at the die, % motor torque, SME and mean residence time of extruded snack according to the independent variables of barrel temperature (X_1), screw speed (X_2) and feed moisture content (X_3)

Parameter	Parameter coefficients [a]				
	Product temperature at the die	Pressure at the die (Pa)	% Motor torque	SME (kJ/kg)	t_m (s)
Intercept	-154.3541	-1565.2083	287.5688	1940.9411	341.4385
Temperature (X_1)	3.7250	23.3500	-2.2188	-18.8402	-0.2050
Screw speed (X_2)	0.4817	6.7333	**-0.4833**	-0.3835	-0.2433
Moisture (X_3)	-7.5625	55.8750	-1.6875	-23.9855	-18.0544
Temperature×Temperature (X_1^2)	0.0092	-0.0483	0.0041	0.0451	0.0141
Temperature×Screw speed (X_1X_2)	-0.0001	-0.0233	0.0017	0.0069	**-0.0062**
Screw speed×Screw speed (X_2^2)	0.000193	0.0056	**0.0003**	0.0011	0.0006
Temperature×Moisture (X_1X_3)	0.0000	-0.4250	0.0225	0.1661	-0.1132
Moisture×Screw speed (X_2X_3)	-0.0200	-0.3067	0.0025	**-0.0308**	**0.0337**
Moisture×Moisture (X_3^2)	0.2708	0.4167	-0.9063	0.1300	0.6264
R^2	0.97	0.90	0.97	0.95	0.94
Model lack of Fit	0.24	0.31	0.48	0.36	0.85

[a] Bold numbers are significant ($p \leq 0.05$) to the regression model



(A)



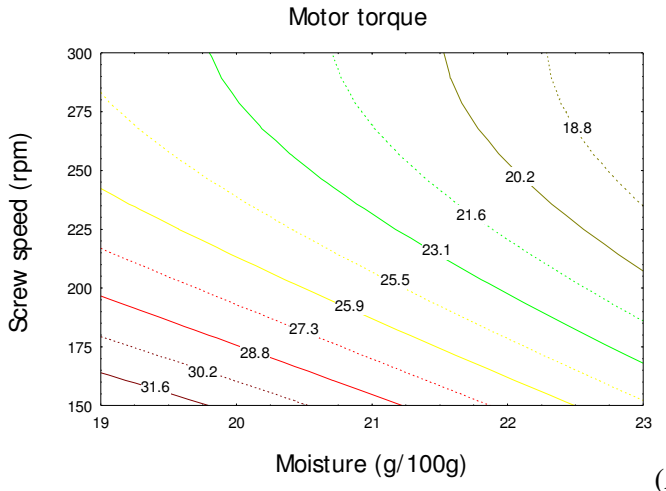
(B)

Figure 10 Contour plots of screw speed and feed moisture to product temperature (°C) (A) and pressure at the die (Pa $\times 10^6$) (B) at the barrel temperature of 135 °C

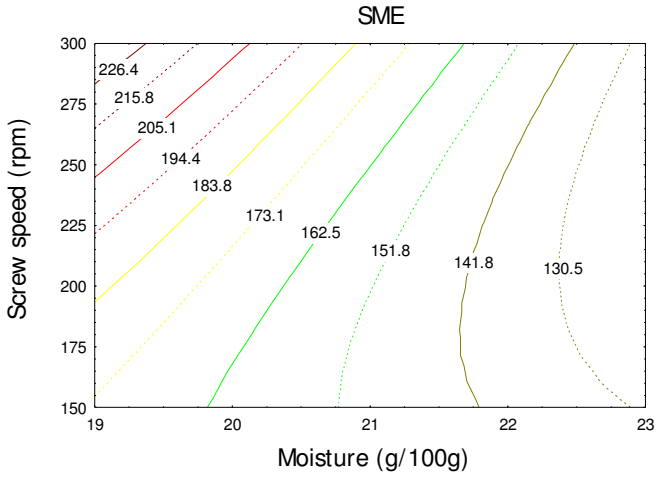
2.1.3. Motor Torque

According to Fichtali and van de Voort (1989), the torque provides information about the amount of energy absorbed by the material due to shear exerted by screws. In this experiment percent motor torque during extrusion ranged between 17-34% depending on feed moisture, screw speed and barrel temperature (Table 14). The lowest motor torque (17%) was obtained at intermediate barrel temperature (135°C), high screw speed (300 rpm) and high feed moisture (23%). The highest torque was observed at intermediate barrel temperature (135°C), low screw speed (150rpm) and low feed moisture (19%). Analysis of the regression model for torque showed the negative linear regression coefficients suggest negative effects (or decreasing influence) of barrel temperature, screw speed and feed moisture on the motor torque (Table 15).

The results indicated that increased barrel temperature, feed moisture and, especially, screw speed decreased motor torque. This is consistent with the observations by Frame (1994) that motor torque and pressure at the die are influenced by the screw speed. Motor torque and pressure at the die are also functions of the dough viscosity during extrusion cooking. Most food doughs show pseudoplastic behaviour (usually thixotropic) during extrusion, and a linear relationship has often been observed between screw speed and torque or pressure at the die. The contour plots for torque at 135°C is shown in Figure 11-A. The figure reveals that torque decreased with increasing moisture and screw speed. It also shows the non-linear influence of screw speed on the torque. The decrease in motor torque with increased screw speed is thought to be associated with decreased barrel fill which decreases the resistance against the melt viscosity through shear thinning, temperature increase and molecular break down. The decrease in melt viscosity provides less resistance to screw rotation (Lu *et al*., 1992). Moisture is also a known plasticizer that influences the viscosity of food doughs. High feed moisture usually provides less viscosity than low feed moisture content (Suknark *et al*., 1998).



(A)



(B)

Figure 11 Contour plots of screw speed and feed moisture to motor torque (%) (A) and and SME (kJ/kg) (B) at the barrel tempearture of 135 °C

Motor torque is a very sensitive indicator of steady operation during extrusion. Fluctuation of motor torque usually indicates a non-steady state of extrusion conditions, and it occurs when there is erratic feeding and, surging and causes plugging of the die. In this study, motor torque fluctuations were observed at high barrel temperature (145°C) and low feed moisture (19-21%) that resulted in wide variations in recorded values for various process parameters.

2.1.4 Specific Mechanical Energy (SME)

Percent motor torque, pressure at the die, and SME are closely related. SME is directly affected by the changes of the screw speed and feed rate (Fichtali and van de Voort, 1989). The SME is related to the degree of product transformation, and it affects extrudate properties such as expansion, density and other geometric and textural characteristics (Iwe *et al*., 2001). The SME from this study at a material feed rate of 10 kg/h (db) ranged between 126-234 kJ/kg. The highest SME was observed at high screw speed and low feed moisture. The predictive regression coefficient showed negative relationships of temperature and feed moisture and a positive relationship between screw speed and SME. The contour plots for SME (Figure 11-B) shows that increasing screw speed increased SME, while increasing feed moisture reduced it. Similar results were reported by Akdogan (1996).

2.1.5 Mean residence Time and Residence Time Distribution

Mean residence time (t_m) and residence time distribution (RTD) reflects the mean time duration and the pattern for the material to transit through the extruder barrel. It is a distribution because of the transport mechanism (resulting in a net forward flow) through the barrel, resulting in various degrees of spread instead of plug flow. It represents the time that the material is exposed to the heat, shear and other chemical reactions that occur in the extrusion barrel. Knowledge of RTD is important for understanding nutrient degradation, food safety and product quality (Peng *et al*., 1994).

t_m in this study ranged from 83-111 s (Table 14). Longer t_m were observed at lower screw speed (Figure 12 and 13). The cross products of barrel temperature and screw speed and screw speed and feed moisture showed significant effects ($p \leq 0.05$) on the model for mean residence time (Table 15). Thus, all the extrusion variables of barrel temperature, screw speed and feed moisture significantly influenced the mean residence time.

1.) Effect of temperature on t_m and RTD

The results show that increasing barrel temperature caused a reduction in pressure at the die, motor torque, and mean residence time. It was surmised that these effects were probably due to lowering of the dough viscosity as the barrel temperature was increased. Extrusion at barrel temperature of 125°C at 225 rpm and 23% moisture showed a t_m of 102.3 s (Table 14). The mean residence time was reduced to 96.9 s when the extrusion temperature was raised to 145°C at the same screw speed (225 rpm) and moisture content (23%). Temperature did not show much effect on the spread of the RTD as shown by the E(t) and F(t) curves (Figure 14-A and B) (E(t) is a function of exit age time and F(t) is an function of the cumulative exit age time.). The model for t_m shows a significant interaction between temperature and screw speed. The contour plot for t_m as a function of a screw speed and temperature shows that at a given screw speed, increasing temperature reduced the t_m (Figure 12). At low screw speed, however, increasing temperature increased the t_m at low moistures.

2.) Effect of screw speed on t_m and RTD

Increasing screw speed showed linear and non-linear reduction in t_m, depending on feed moisture and barrel temperature. When screw speed was increased from 150 to 300 rpm at 125°C and 21% feed moisture, the t_m decreased from 105.6 to 98.0 seconds (Figure 15-A). Plots of the E (t) and F (t) functions are shown in Figure 15-A and B. The E(t) curve was sharper and shifted to the left at higher screw speeds (Figure 15-A), indicating that under those extrusion

conditions there was less spread in RTD as it approached plug flow, and the t_m was shorter. There was more spread in RTD of the material at lower screw speeds, resulting in longer t_m. Similar observations were found by Gogoi and Yam, 1994; Yeh *et al*., 1992 and Unlu and Faller, 2002.

The contour plots of the regression model for t_m as a function of screw speed and barrel temperature (Figure 12) and screw speed and feed moisture (Figure 13) show that increasing the screw speed reduced t_m. Similar observations were reported by Unlu and Faller (2002) and Ainsworth *et al*., (1997). Lee and McCarthy (1996) found the screw speed was highly significant to the t_m for rice extrudates. The effects of increased screw speed on residence time reduction might be due to decreased degree of barrel fill (which was not determined in this study) and the shear thinning properties of food material (Yeh *et al*., 1992).

3.) Effect of moisture on t_m and RTD

Increased feed moisture at constant barrel temperature and screw speed increased t_m. The t_m at 19 % and 23% moisture at 135°C and 300 rpm were 87.4 and 99.2 seconds, respectively. The E(t) and the F(t) curves for low and high feed moisture extrusion at constant temperature (135°C) and screw speed (300 rpm) were similar in shape with little difference in RTD spread (Figure 16-A).

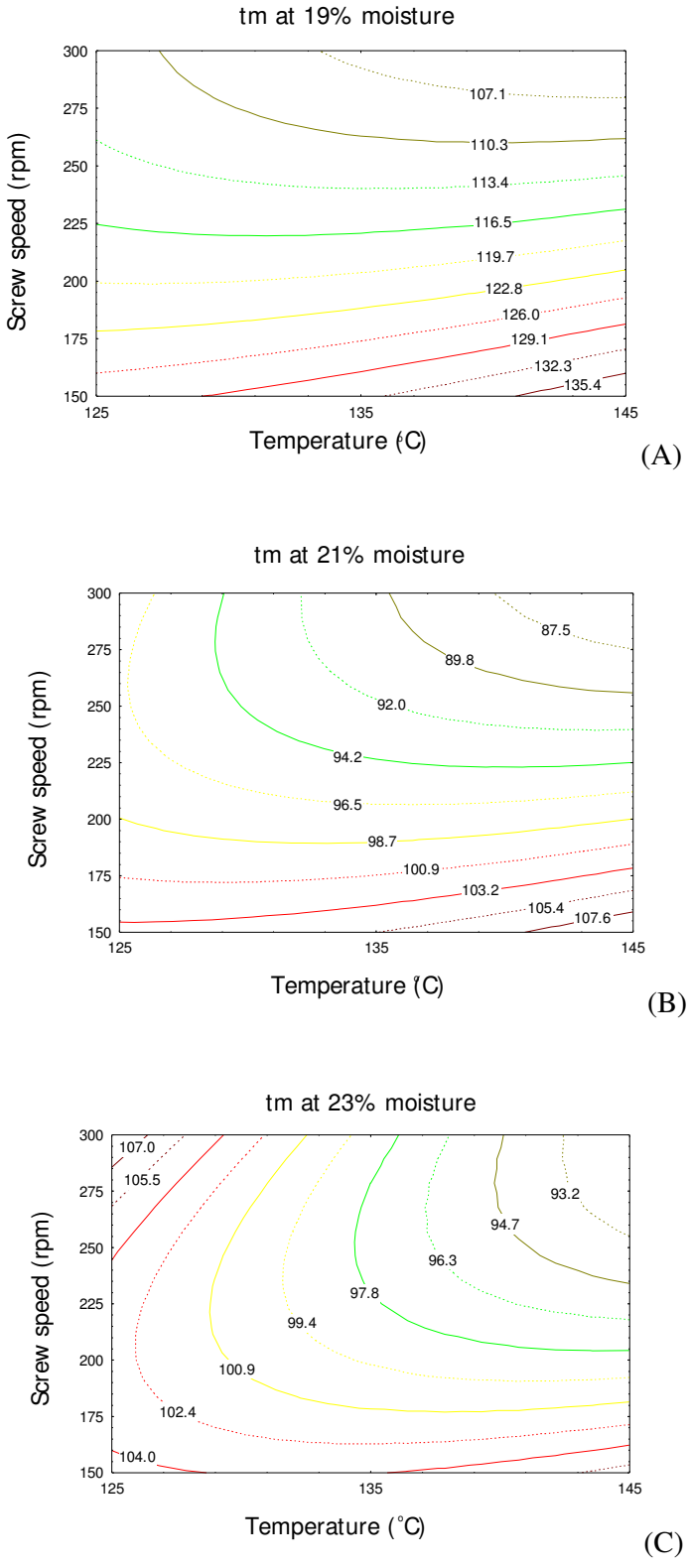
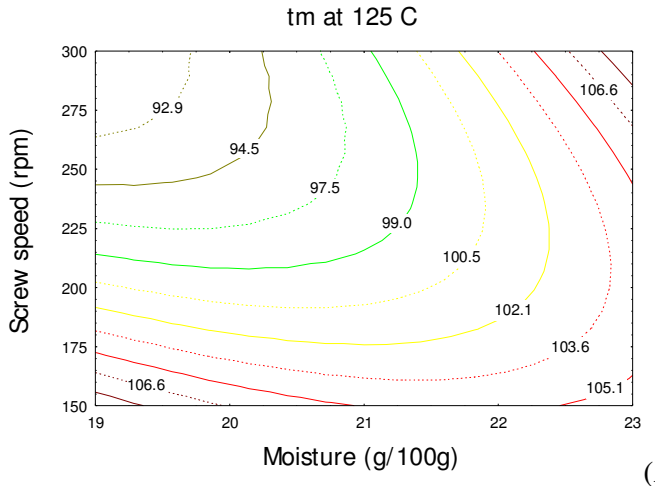
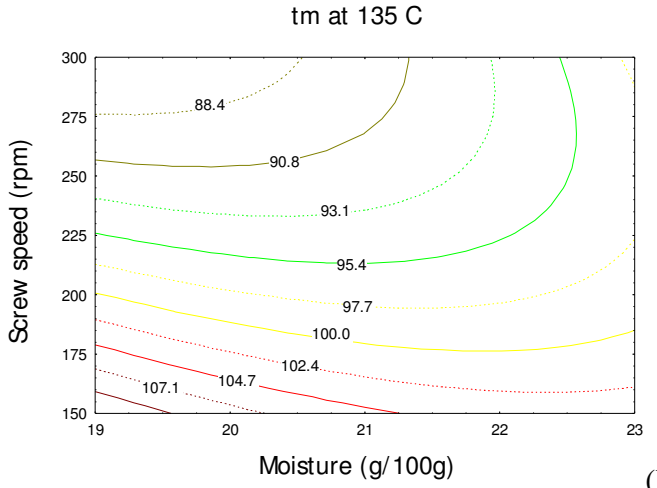


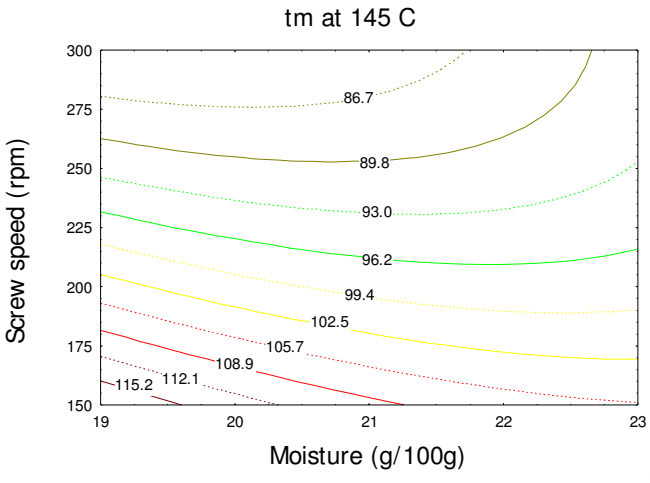
Figure 12 Contour plots of screw speed and barrel temperature to t_m (s) at the feed moisture content of 19% (A), 21% (B), and 23% (C)



(A)

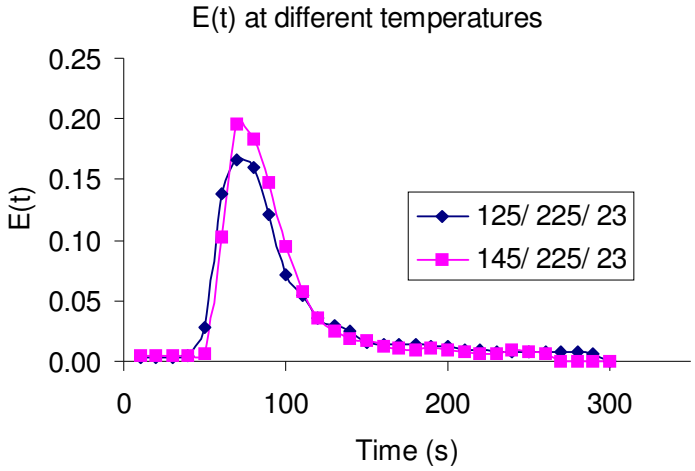


(B)

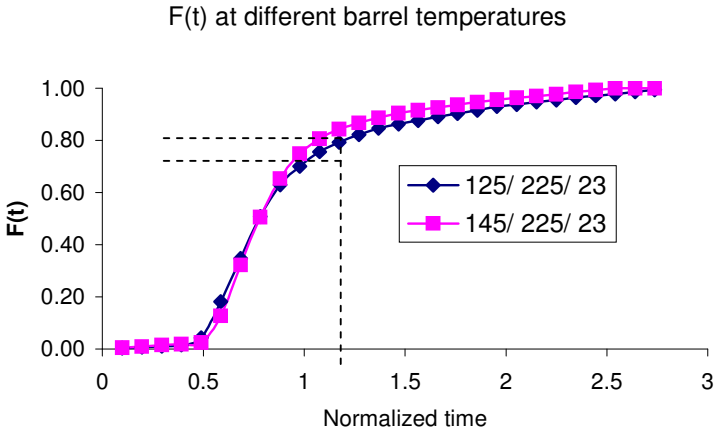


(C)

Figure 13 Contour plots of screw speed and feed moisture to t_m (s) at the barrel temperature of 125 (A), 135 (B), and 145 °C (C)

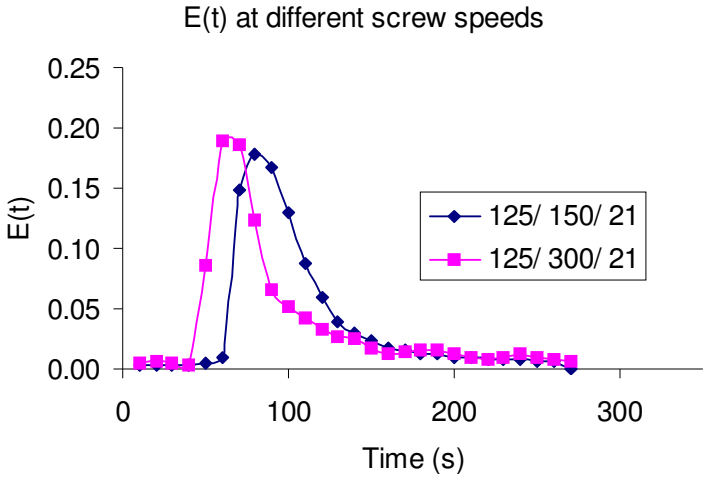


(A)

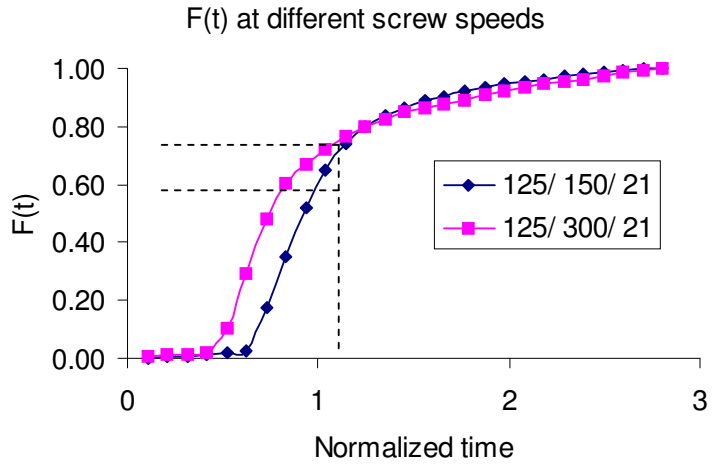


(B)

Figure 14 E(t) (A) and F(t) (B) plots of the residence time distribution at different temperatures (◆ 125°C, ▪ 145°C)

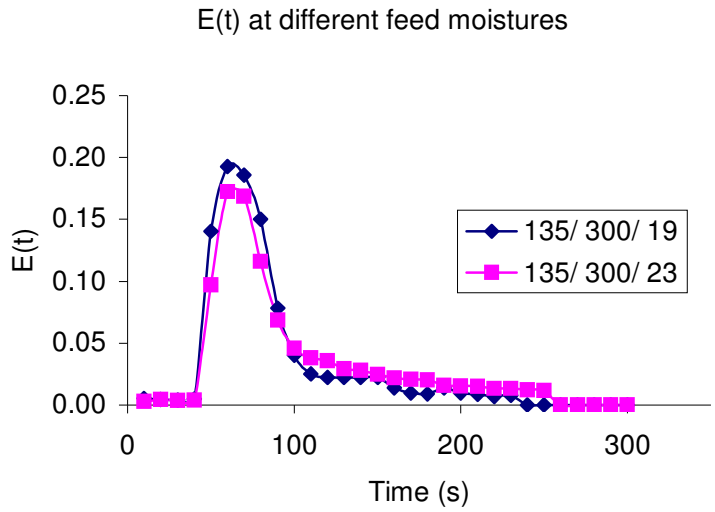


(A)

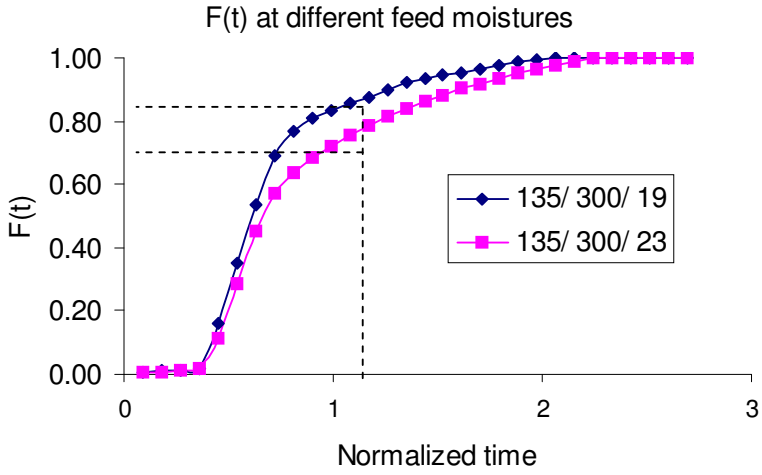


(B)

Figure 15 E(t) (A)and F(t) (B) plots of the residence time distribution at different screw speeds(◆ 150 rpm, ▪ 300 rpm)



(A)



(B)

Figure 16 E(t) (A)and F(t) (B) plots of the residence time distribution at different feed moisture (◆ 19 g/100g, ▪ 23 g/100g)

2.2 Moisture and Fat Content of the Extrudates

The moisture and fat contents in extrudates are shown in Table 16. After extrusion, moisture contents in all extrudates from 15 runs after drying at 80°C for 10 min were 4.48-7.54% depended upon the feed moisture contents. Moisture content was slightly affected by temperature and screw speed (Figure 17). Moisture levels of the extruded snacks are normally between 8 and 12 % and require additional drying to impart the desired final product texture and mouth feel (Rokey, 2000). Moisture loss during extrusion is due to the vaporization of water into steam during the rapid pressure loss at the die opening. When the dough exits the extruder through the die opening, the superheated water is exposed to atmospheric pressure. This rapid pressure loss causes stretching and expansion of the starch matrix (Moore, 2000). Feed moisture content influences the degree of gelatinization occurring during extrusion which affects the physical characteristics of the extrudates.

Jager *et al*. (1991) reported that lipids added to extrusion mixtures must be thoroughly mixed before extrusion to avoid oil separation. Oil also lubricates the screw and barrel surfaces, therefore, reducing the friction factor. Addition of lipid in small quantity (<3%) in extrusion cooking has little effect on the extrudate expansion at the die, while amounts over 5% decrease extrudate expansion (Harper, 1992). Added corn oil up to 4% to maize grits showed increased expansion (Mohamed, 1990).

Pan *et al*. (1992) reported that addition of soybean oil (3%) to rice flour was the optimum level for expansion and crispiness. Park *et al*. (2001) studied single screw extrusion of defatted soy flour, corn starch and raw beef blends containing up to 5% fat. They found that the optimal fat content of the blends for most product properties (expansion ratio, bulk density, shear force, water sorption and color) were at fat levels of 2.5-3.0%. In this study, about 1.78% (db) of refined menhaden oil was added so that the total fat content from fish powder and menhaden oil in the mixture was approximately 4.3% (db). The extrudates contained 4.1-5.5% of fat content according to analysis (Table 16).

Table 16 Moisture and fat content of the extrudates

Run	Experimental condition[a]			Moisture content (%)[b]	Fat content (%)[c]
	Barrel temperature[a] (°C)	Screw speed (rpm)	Moisture (g/100g)		
RM[c]	-	-	-	8.3±0.03	4.3±0.07
1	125	225	23	7.5±0.08	4.4±0.37
2	125	150	21	7.3±0.07	4.5±0.29
3	125	300	21	6.7±0.06	4.4±0.00
4	125	225	19	4.8±0.11	4.7±1.08
5	135	150	23	7.6±0.11	4.4±0.03
6	**135**	**225**	**21**	**6.0±0.00**	**4.4±0.03**
7	**135**	**225**	**21**	**5.6±0.05**	**4.5±0.13**
8	**135**	**225**	**21**	**5.7±0.12**	**4.1±0.89**
9	135	300	23	6.9±0.11	4.4±0.65
10	135	150	19	5.2±0.08	4.1±0.89
11	135	300	19	4.5±0.03	5.5±0.07
12	145	225	23	7.6±0.06	5.5±0.19
13	145	150	21	6.7±0.06	4.8±0.21
14	145	300	21	5.4±0.03	4.5±0.52
15	145	225	19	5.0±0.10	4.7±0.05

[a] Barrel temperature (°C) of barrel zone 2, 3, and 4

[b, c] Means of three measurements and calculated as % dry basis (db).

[d] RM = raw material mixture

2.3 EPA, DHA and Vitamin E Retentions

EPA and DHA in the samples originated from the fish powder and added menhaden oil. Menhaden oil was added to increase EPA and DHA to the desirable n-3 fatty acid content of 900mg/100g sample. The use of EPA and DHA as a dietary supplements is safe and lawful under 21 C.F.R. § 101.14, provided daily intakes of EPA and DHA n-3 fatty acid do not exceed 3 g/person/day from combined intake of conventional food and dietary supplements (FDA/CFSAN, 2000). Vitamin E (mixed tocopherol concentrate) was added as an antioxidant. Eicosapentaenoic acid, DHA and EPA+DHA contents and their respective retentions in products after extrusion under varied conditions and subsequent drying are shown in Table 17.

2.3.1 EPA and DHA Retentions

Expected EPA and DHA contents in the raw material calculated from EPA and DHA contents in all ingredients were 381 mg/ 100g and 544 mg/100g, respectively. After extrusion, EPA and DHA contents were 278-358 mg/100g (71-94% retentions) and 433-591 mg/100g (80-108 % retention), respectively. The EPA+DHA contents were 702-948 mg/100g (80-102% retention). The highest EPA+DHA retention was found at 145°C barrel temperature, 225 rpm screw speed and 23% feed moisture.

2.3.2 Vitamin E Retentions

Vitamin stability varies with vitamin structure, extrusion conditions, and food matrix compositions (Camire, 1998). After extrusion, α-, β-, γ-, δ-tocopherols and α, γ and δ-tocotrienols were present in all extrudates samples. The content of individual tocopherols and tocotrienols in the extrudates are shown in Table 18.

In the extrusion formulation, α-tocopherol was found in the highest amount followed by γ-, δ-, β-tocopherol (2.45, 1.00, 0.53 and 0.03 mg/100g), respectively. Alpha-tocopherol retention for all extruded samples ranged from 69.3-78.9% except at the extrusion temperature of 135°C, 300rpm screw speed and 23% moisture, where the retention was more than 90.0%. For γ- and δ- tocopherols, the retentions ranged from 57.4-62.9% and 55.0-63.3%. Alpha-tocotrienol was not found in any samples. Gamma and δ-tocotrienols recoveries in some samples were higher than the calculated number but not higher than the actual number in raw material that was analyzed at the same time with others. Total tocopherol and tocotrienol retention ranged from 54.7-76.9%.

Regression models were developed to predict the effects of the independent variables (temperature, screw speed and feed moisture content) on the response variables (EPA, DHA, EPA+DHA and vitamin E). The model for EPA (Table 19) had R^2 of 0.87 and showed no lack of fit. It suggests that the model is adequate. The linear effects of temperature (X_1), the quadratic effect of temperature (X_1^2), screw speed (X_2^2) and moisture (X_3^2) were significant ($p \leq 0.05$) in the EPA regression model. The model for DHA and EPA+DHA (Table 18) had R^2 of 0.59 and 0.69, accordingly. Both models showed no model lack of fit, and, thus, the models adequately fit the experimental data. However, none of the individual regression coefficients in DHA and EPA+DHA regression models were significant.

Table 17 Effect of extrusion conditions on EPA and DHA

Extrusion conditions			EPA		DHA		EPA+DHA	
Barrel Temperature (°C)	Screw speed (rpm)	Moisture (g/100g)	Content [b] (mg/100g)	Retention[c] (mg/100g)	Content [b] (mg/100g)	Retention[c] (mg/100g)	Content [b] (mg/100g)	Retention[c] (mg/100g)
Raw material (Calculated value) [a]			381	100.0	544	100.0	925	100.0
125	225	23	278±20	72.9±5.3	482±38	88.6±7.0	760±58	82.1±6.3
	150	21	303±16	79.7±4.2	528±30	97.1±5.5	832±46	89.9±5.0
	300	21	308±20	80.8±5.2	548±27	100.7±4.9	856±46	92.5±5.0
	225	19	269±16	70.5±4.2	433±38	79.6±7.0	702±54	75.9±5.8
135	150	23	332±62	87.1±16.4	568±45	104.4±21.1	900±178	97.3±19.2
	225	21	310±4	81.4±1.0	528±4	97.1±0.8	839±6	90.6±0.7
	225	21	286±5	75.1±1.3	487±10	89.4±1.9	773±15	83.5±1.6
	225	21	314±49	82.5±12.8	537±83	98.8±15.3	852±32	92.1±14.3
	300	23	314±36	82.3±9.3	540±47	99.2±8.6	854±82	92.0±8.9
	150	19	296±11	77.5±2.8	500±24	92.0±4.4	796±35	86.1±3.8
	300	19	356±3	93.4±0.8	586±16	107.6±3.0	942±27	101.8±1.9
145	225	23	358±10	93.8±2.7	590±25	108.5±4.6	948±35	102.5±3.8
	150	21	290±10	76.1±2.7	470±14	86.4±2.6	760±24	82.2±2.6
	300	21	280±43	73.4±11.2	461±83	84.7±15.2	741±125	80.1±13.6
	225	19	287±25	75.3±6.5	458±54	84.3±9.9	745±79	80.5±8.5

[a] Calculated from the composition of the ingredients

[b] Mean of 4 replications

[c] % retention is based upon raw material composition

Table 18 Tocopherols and tocotrienols in extrudates.

Run	Tocopherols and tocotrienols (mg/100g) [a, b, c]							Total tocopherols and tocotrienols (mg/100g)	Total tocopherols and tocotrienols Retention (%)
	α-T[a]	β-T	γ-T	δ-T	α-T3	γ-T3	δ-T3		
Calculated amount	2.38	0.01	1.08	0.60	0.07	0.15	0.02	4.31	100.0
Raw Material	2.45	0.03	1.00	0.53	ND[d]	0.25	0.02	4.28	99.2
1	1.64 (68.9)	0.03	0.62 (57.4)	0.33 (55.0)	ND	0.17 (62.0)	0.02	2.36	54.7
2	1.66 (69.7)	0.03	0.63 (58.3)	0.34 (56.7)	ND	0.18 (72.0)	0.01	2.52	58.4
3	1.75 (73.5)	0.03	0.67 (62.0)	0.38 (63.3)	ND	0.19 (76.0)	0.02	2.57	59.6
4	1.71 (71.8)	0.03	0.67 (62.0)	0.36 (60.0)	ND	0.18 (72.0)	0.02	2.55	59.1
5	1.77 (71.8)	0.03	0.67 (62.0)	0.34 (56.7)	ND	0.18 (72.0)	0.02	2.52	58.6
6	1.71 (71.8)	0.03	0.67 (62.0)	0.34 (56.7)	ND	0.19 (76.0)	0.03	2.52	58.4
7	1.83 (76.9)	0.03	0.69 (63.9)	0.38 (63.3)	ND	0.18 (72.0)	0.02	3.02	69.9
8	1.65 (69.3)	0.02	0.63 (58.3)	0.35 (58.3)	ND	0.17 (62.0)	0.03	2.77	64.3
9	2.17 (91.2)	0.04	0.69 (63.9)	0.37 (61.7)	ND	0.18 (72.0)	0.03	3.32	76.9
10	1.65 (69.3)	0.03	0.63 (58.3)	0.35 (58.3)	ND	0.17 (62.0)	0.02	2.76	63.9
11	1.71 (71.8)	0.03	0.65 (60.2)	0.37 (61.7)	ND	0.18 (72.0)	0.03	2.89	67.0
12	1.88 (78.9)	0.03	0.69 (63.9)	0.38 (63.3)	ND	0.19 (76.0)	0.03	3.04	70.6
13	1.85 (77.7)	0.03	0.69 (63.9)	0.39 (65.0)	ND	0.18 (72.0)	0.03	3.05	70.7
14	1.75 (73.5)	0.03	0.66 (61.1)	0.38 (63.3)	ND	0.18 (72.0)	0.02	2.93	67.9
15	1.66 (69.7)	0.03	0.65 (60.2)	0.36 (60.0)	ND	0.17 (62.0)	0.02	2.81	65.2

[a] numbers in parentheses represent % retention

[b] α,β, γ, δ-tocopherols and α, γ, δ-tocotrienols

[c] the numbers were corrected with % α, γ, δ-tocopherols and α, γ, δ-tocotrienols recoveries

[d] "ND" = not detected; vitamin E homolog is below limit of detection: 0.265, 0.0525, 0.0108 and 0.0562 ng/ 20 µl for α, β-, γ, δ-tocopherols

Contour plots generated from regression models show the effects of extrusion conditions on EPA, DHA and EPA+DHA contents. The contour plot for EPA (Figure 18-A) has a saddle shape with highest retentions at high moisture and low screw speed and low moisture and high screw speed (significant quadratic effects ($p \leq 0.05$) exist for the various extrusion parameters). This indicates that increasing screw speed at low feed moisture and increasing feed moisture at low screw speed increased EPA retention. There were no significant interactions between any independent variables indicating that the effects of screw speed and feed moisture on EPA content would be similar at any barrel temperature. However, higher EPA contents were found at an extrusion temperature of 135°C compared to 125°C and 145°C at the same screw speed and feed moisture content (figures are not shown). The contour plots for DHA (Figure 17-B) and EPA+DHA (Figure 17-C) contents show similar trends.

The regression model for total vitamin E (Table 19) had R^2 of 0.74 with no model lack of fit. However, none of the individual regression coefficients were significant in the model. The contour plot generated from the predictive regression model for total vitamin E (Figure 18) shows the effect of feed moisture and barreltemperature when screw speed is constant. Increased levels of feed moisture and temperature increased retention of vitamin E at any screw speed. Cheftel (1986) noted that an increase in screw speed at low moisture content probably enhanced vitamin destruction due to higher shear force leading to increased temperature. Higher screw speed at higher moisture contents might decrease the vitamin destruction due to shorter residence time. In this study, residence time was shortened when screw speed increased and moisture content decreased.

Table 19 Coefficients of parameters in the regression models for EPA, DHA and EPA+DHA and vitamin E in extruded snack according to the independent variables of barrel temperature (X_1), screw speed (X_2) and feed moisture content (X_3)

Parameter	Parameter coefficients [a]			
	EPA	DHA	EPA+DHA	Vitamin E
Intercept	**-7001.7201**	-2083.7385	-9085.4606	2.83079
Temperature (X_1)	**87.4530**	61.5515	149.0045	0.1751
Screw speed (X_2)	2.2638	4.2353	6.4983	-0.0216
Moisture (X_3)	112.9575	-205.9119	-92.9544	-1.0769
Temperature×Temperature (X_1^2)	**-0.2850**	-0.3116	-0.9567	-0.0009
Temperature×Screw speed (X_1X_2)	-0.0100	-0.0159	-0.0259	-0.000056
Screw speed×Screw speed (X_2^2)	**0.0041**	0.0039	0.0082	0.000016
Temperature×Moisture (X_1X_3)	-0.4059	1.2508	0.8449	0.0052
Moisture×Screw speed (X_2X_3)	-0.13169	-0.1838	-0.3154	0.0011
Moisture×Moisture (X_3^2)	**-0.7268**	2.1455	1.4194	0.0031
R^2	0.87	0.59	0.69	0.75
Model lack of Fit	0.53	0.18	0.29	0.63

[a] Bold numbers are significant ($p \leq 0.05$) to the regression model ($p \leq 0.05$)

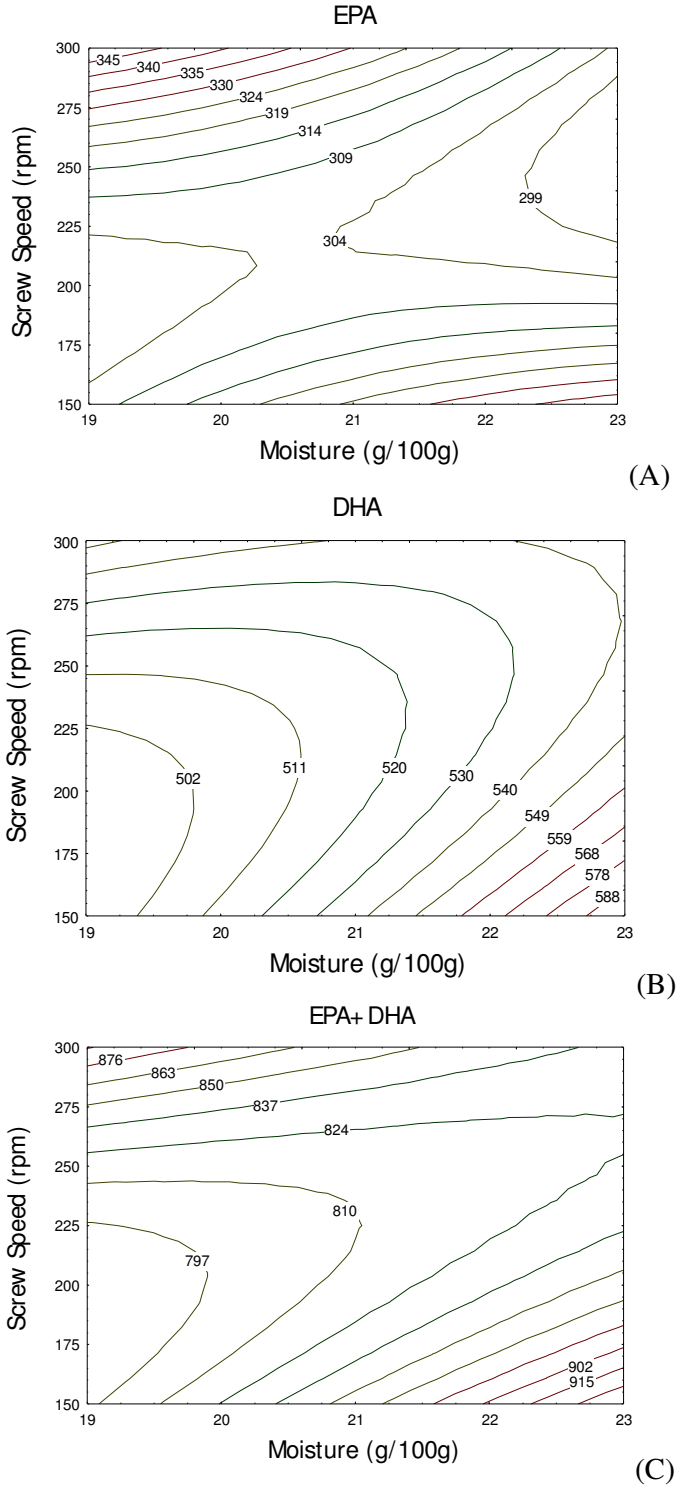
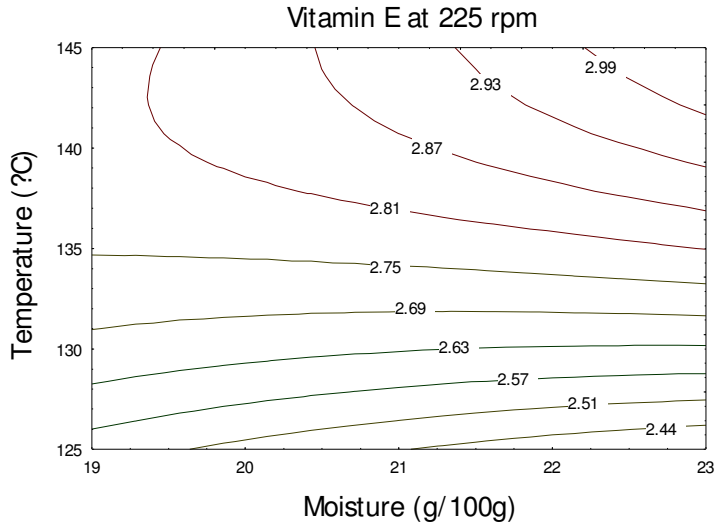
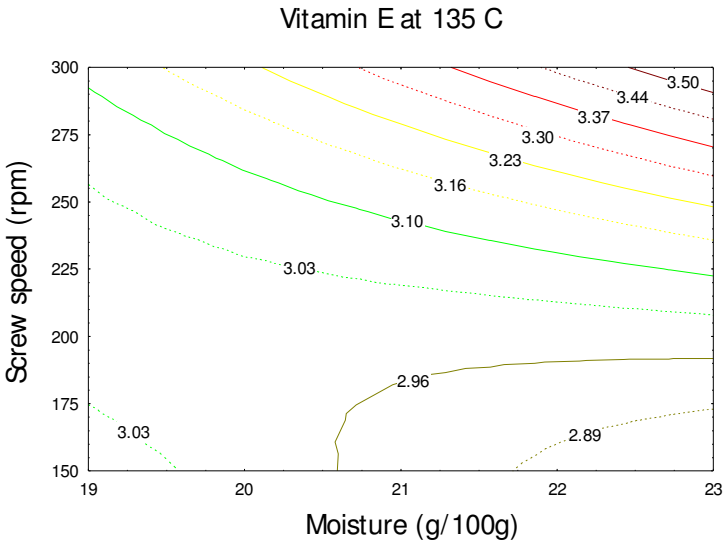


Figure 17 Contour plots of the effects of screw speed and raw material moisture on EPA (A), DHA (B) and EPA+DHA (C) in extrudates (mg/100g) at the barrel temperature of 135°C



(A)



(B)

Figure 18 Contour plots of the effects of barrel temperature and feed moisture on vitamin E in extrudates (mg/100g) at the screw speed of 225 rpm (A) and the effects of screw speed and feed moisture at the barrel temperature of 135°C (B)

Physical and chemical properties of extrudates are the result of

temperature, pressure and intense mechanical shear effects of extrusion cooking (Shin *et al*., 1997). Chemical changes produced by extrusion cooking include thermal degradation, depolymerization and recombination of fragments. Vitamin stability varies with vitamin structure, extrusion conditions and food matrix compositions. In extrusion studies, usually 2-3 extrusion parameters are examined. These may include screw speed, screw configuration, feed rate, die geometry, barrel temperature and feed moisture which influence shear, product viscosity in the barrel and residence time. Secondary extrusion parameters include specific mechanical energy and product or mass temperature (Camire, 1998). Degradation of vitamins depends on factors encountered during food processing and storage such as temperature, oxygen, light, moisture, pH and exposure time. In general, the retention of vitamins during extrusion decreases at higher barrel temperature and screw speed. Increasing moisture and throughput increases vitamin retention by reducing mass temperature and limiting thermal degradation (Killeit, 1994).

Tocopherols and tocotrienols are effective inhibitors of lipid oxidation. The antioxidant activity of the tocopherols and tocotrienols is due to their ability to donate their phenolic hydrogens to lipid free radicals. The relative antioxidant activity *in vivo* is in the order of α- > β- > γ- > δ-tocopherols. The reverse order was obtained when the relative antioxidant potencies were compared *in vitro* in fats, oils, and lipoproteins. However, it should be noted that the activities of tocopherols *in vitro* areaffected by temperature, light, type of substrate, solvent, pro-oxidants and synergists.

The antioxidant activity of tocopherols was reported to be in the order of α- > β- > γ- > δ- at mild temperatures and reversed order at high temperatures (Kamal-Eldin and Appelqvist, 1996). However, at higher temperatures, α-tocopherol has a lower pro-oxidant effect. This phenomenon may be related to the lower solubility of oxygen in oils at high temperature (Marinova and Yanishlein, 1992).

2.4 Effects of Extrusion Conditions on Physical Properties (product density, expansion ratio and shear strength) of the Extrudates

Extrusion is a complex process which requires close control of many variables to consistently obtain desired product attributes. Process variables such as temperature, screw speed, throughput, feed composition and moisture content, along with screw configuration and die geometry, affect mechanical and thermal energy inputs and residence times. These system variables, also known as intermediate process variables, induce reactions that affect nutritional value, texture, flavor, color and microbial quality (Choudhury and Gautam, 2003). Gogoi *et al*. (1996 a, b) showed that incorporation of fish protein into starch-rich ingredients, such as rice flour, significantly decreased mechanical energy input, resulting in products with reduced expansion and increased hardness.

The puffing or expansion of direct expanded snack products is created by heating the ingredients to temperatures over 100°C. During the extrusion cooking of biopolymers, the viscoelastic material is forced through a die (Padmanabhan and Bhattachayrya, 1989). Inside the extruder barrel, water in the dough mass remains liquid because the dough is under pressure. As the dough exits the extruder through the die opening, the super heated water is vaporized into steam, causing stretching and expansion of the starch matrix which produces an expanded, porous structure (Frame, 1994). Expansion of starch affects the texture and structure of the finished products (Chen and Yeh, 2001). In general, the characteristics of animal protein extrudates are affected by moisture and protein content (Areas, 1992). A lower bulk density and higher expansion ratio indicates greater puffing or a more highly expanded product. Puffing is directly related to temperature and inversely related to feed moisture of snack-like cowpea extruded products (Falcone and Phillips, 1988).

Product moisture, product density, expansion ratio and shear strength of the extrudates are shown in Table 20. The regression models for product moisture, product density, expansion ratio and shear strength were characterized high R^2 of 0.84, 0.94, 0.76 and 0.96, respectively. None of the models showed significant lack of fit,

showing the models are adequate (Table 21). Figure 19 shows the apparent effects of screw speed and raw material moisture content on product moisture, product density and expansion ratio at a barrel temperature of 135°C.

2.4.1 Product Moisture

Moisture in the extruded products after drying ranged from 4.5 to 7.6%. The lowest moisture content in the product was found when product was extruded at intermediate barrel temperature (135°C), high screw speed (300 rpm) and low feed moisture (19%). The highest product moisture content was found at intermediate barrel temperature (135°C), low screw speed (150 rpm) and high feed moisture (23%) (Table 20). The models showed a high R^2 of 0.83, and non-significant ($p>0.05$) model lack of fit (Table 21). The negative linear regression coefficient of barrel temperature suggested that increased barrel temperature decreased the moisture of the extrudates, as would be expected (Table 21). In contrast, the regression coefficients of screw speed and, especially, feed moisture showed positive linear relationships with final product moisture levels. However, the quadratic regression coefficients of barrel temperature and screw speed had contrasting effects to the their linear effects. Therefore, the contour plot of the model implies that an increase in screw speed reduced product moisture while increased feed moisture produced products with higher moisture (Figure 19-A).

During extrusion, moisture content depends, in part, on the initial level of moisture in the feed material. Moisture combined with heat contributes to starch gelatinization and provides the aqueous environment necessary for chemical reactions. The amount of water required for starch gelatinization depends on the characteristics of the feed material and the extrusion conditions. Water is also a plasticizer and lubricant in extrusion cooking. It affects rheological properties of the dough and, thus, the transfer of mechanical and thermal energy. As moisture content increases, melt viscosity decreases. Therefore, at high moisture levels and low melt viscosity, the specific mechanical energy (SME) input decreases, resulting in a lower motor torque and lower product temperature (Moore, 1994).

Table 20 Product moisture, product density, expansion ratio and shear strength of the extrudates

Experimental condition			Product moisture[b] (g/100g) db	Product density[c] (g/cm^3)	Expansion ratio[d]	Shear strength[e] (N/cm^2)
Barrel temperature[a] (°C)	Screw speed (rpm)	Moisture (g/100g)				
125	225	23	7.5±0.08	0.40±0.031	2.22±0.062	314±22.0
	150	21	7.3±0.07	0.42±0.018	2.20±0.040	374±12.0
	300	21	6.7±0.06	0.35±0.034	2.25±0.119	256±11.3
	225	19	4.8±0.11	0.32±0.031	2.42±0.108	224±13.3
135	150	23	7.6±0.11	0.41±0.038	2.18±0.096	360±9.0
	225	21	6.0±0.00	0.36±0.019	2.33±0.057	257±13.8
	225	21	6.9±0.11	0.37±0.036	2.32±0.075	287±18.1
	225	21	5.7±0.12	0.32±0.017	2.34±0.057	253±13.4
	300	23	5.3±0.08	0.36±0.024	2.42±0.107	241±18.5
	150	19	5.6±0.05	0.31±0.028	2.42±0.107	245±10.4
	300	19	4.5±0.03	0.29±0.026	2.51±0.100	201±10.8
145	225	23	7.3±0.02	0.35±0.015	2.32±0.042	248±10.8
	150	21	6.0±0.06	0.34±0.030	2.41±0.074	251±10.7
	300	21	5.4±0.03	0.30±0.024	2.47±0.064	195±16.5
	225	19	5.0±0.10	0.28±0.032	2.47±0.097	187±2.3

[a] Barrel temperature (°C) of barrel zone 2, 3, and 4

[b] Moisture of the products after extrusion and drying at 80°C for 10 min

[c,d] Average value from 10 measurements of extrudates after drying at 60°C for 8 h

[e] Maximum force to break the extrudate by using a Standard Kramer shear cell devided by extrudate cross-sectional area

Table 21 Coefficients of parameters in the regression models for product moisture, product density, expansion ratio and shear strength of extruded snack according to the independent variables of barrel temperature (X_1), screw speed (X_2) and feed moisture content (X_3)

Parameter	Parameter coefficients			
	Product moisture	Product density	Expansion ratio	Shear strength
Intercept	-17.0445	-2.1690	3.238	-3528.9057
Temperature (X_1)	-0.7084	0.0090	-1.143	20.2669
Screw speed (X_2)	0.0574	-0.0010	0.0220	-1.5714
Moisture (X_3)	5.9449	0.1890	0.5680	258.0658
Temperature×Temperature (X_1^2)	0.0031	0.00003	0.0007	-0.07601
Temperature×Screw speed (X_1X_2)	-0.0001	-0.000007	-0.0002	0.0207
Screw speed×Screw speed (X_2^2)	-0.00002	0.0000005	0.00001	0.0018
Temperature×Moisture (X_1X_3)	-0.0069	-0.0002	0.0006	-0.3809
Moisture×Screw speed (X_2X_3)	-0.0021	-0.00004	0.00009	-0.1256
Moisture×Moisture (X_3^2)	-0.0964	-0.0030	-0.0170	-3.7932
R^2	0.84	0.94	0.76	0.96
Model lack of Fit	0.42	0.91	0.07	0.62

Higher temperature provides a higher potential energy for flashing off super-heated water from extrudates as products exit the die. With higher barrel temperatures, extrudates exiting the die lose more water and become lighter in weight. Extrusion of waxy hulless barley indicated that increased extrusion temperature and feed moisture decreased pressure at the die and SME (Köksel *et al*., 2004).

2.4.2 Product Density and Expansion Ratio

Product density and expansion ratio are closely related. Product density from this experiment ranged from 0.280 to 0.415 g/cm^3. The extrusion conditions that produced the lowest and the highest product density were very similar to those producing the lowest and highest product moisture since they are closly related. The regression model showed high R^2 and no significant lack of fit ($p \leq 0.05$). However, none of the individual independent variables were significant. The contour plot shows that product density increases as feed moisture increases. In contrast, increased screw speed decreased the bulk density (Figure 19-B).

The product with the highest radial expansion ratio (2.51) was produced at moderate temperature (135°C), high screw speed (300 rpm) and low feed moisture (19%) (Table 20). The regression model showed high R^2 and no significant lack of fit ($p \leq 0.05$). However, the contour plot (Figure 19-C) suggested that increased feed moisture decreased the radial expansion ratio.

Increased from 150 to 250 rpm decreased radial expansion, whereas increased screw speed over 250 rpm increased radial expansion. However, the product density decreased consistently with increasing screw speed. Decreased radial expansion at screw speed between 150 to 250 rpm with a concomitant decrease in product density is probably due to greater longitudinal expansion (axial) of the product (Figure 19-B and C).

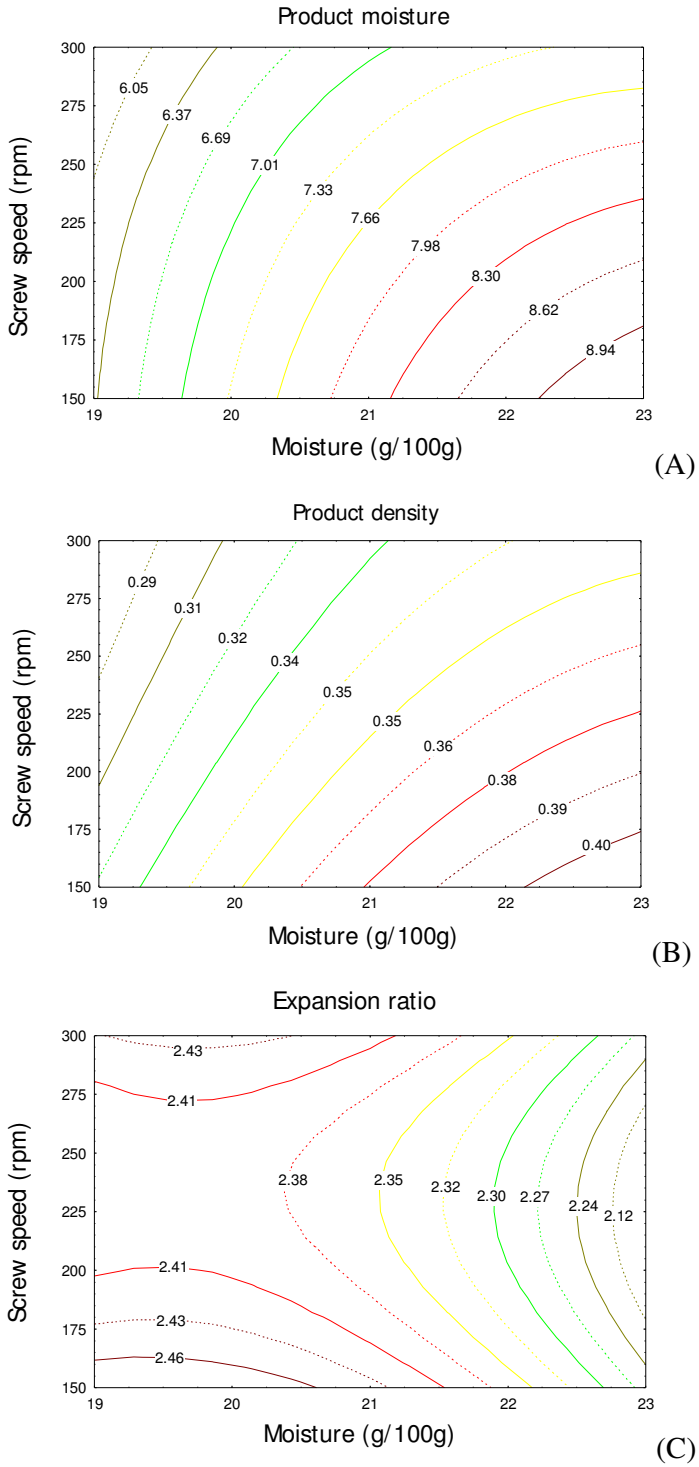


Figure 19 Contour plots of screw speed and feed moisture on product moisture (A), bulk density (g/cm^3) (B), and expansion ratio (C) of the extrudates at the barrel temperature of 135°C

Guha *et al*. (1997) found that screw speeds of 200-300 rpm lowered bulk density of extruded rice flour. The authors reported that the combination of high temperature and high screw speed yields a product with low density. High temperature provides more thermal input, leading to complete gelatinization even at high screw speeds that decrease residence time. Structural break down of protein and starch in the high shear environment also leads to low density products (Guha *et al*., 1997). Bindzus *et al*. (2002) reported that higher SME input increased molecular breakdown of amylopectin in the extruded rice starch. Ilo *et al*. (1996) reported the dough temperature (150-160°C) and feed moisture content (13-17%wb) significantly affected the bulk density of extruded maize grits that extruded from conical counter-rotating twin screw extruder. Ilo *et al*. (1999) also reported that extrudate density is inversely related to overall expansion in rice-flour and amarath blend extrudates extruded at 150-190°C and 11-16% (wb) moisture with the same extruder. Lawton *et al*. (1985) reported that higher extrusion temperatures (130-190°C) increased the expansion of a mixture of wheat, gluten and bran. Higher temperature and lower feed moisture decreased bulk density.

Őzer *et al*. (2004) reported that screw speed and feed moisture significantly affects radial and axial expansion ratios in extruded corn flour snacks. The authors reported that overall expansion increased with increased screw speed and remained constant with changing in feed moisture and feed rate. Radial expansion increased with increased screw speed. Axial expansion increased with increased screw speed and in contrast in radial expansion, decreased as feed moisture increased. As the screw speed increased, the shear applied by reverse pitch elements to the material inside the extruder barrel increased, giving a more developed and uniform dough with better expansion properties at the die exit. Ilo *et al*. (1996) reported that feed moisture had a highly significant effect on the radial expansion ratio. Hashimoto *et al*. (2002) found that the expansion ratio in extruded cassava starch decreased with increased feed moisture content. Fan *et al*. (1994) and Ryu and Ng (2001) stated that lower viscosity of the melt at high feed moisture caused bubble shrinkage and collapse increased. Condensation of water vapor which produces a negative pressure difference for bubble growth which leads to collapse.

Incorporation of reverse screw elements is a an efficient method during twin-screw extrusion to increase shear energy inputs to feed materials to increase product transformation. The degree of conversion of rice flour to gel or melt increased with the number of reverse screw elements (Gogoi *et al*., 1996). Starch gelatinization of extruded maize grits increased with increasing SME during extrusion. The increased degree of starch gelatinization led to more expansion (Ilo *et al*., 1996). Giri and Banyopadhyay (2000) stated that the expansion of the melted dough at the die exit depends upon the extent of gelatinization of starch and the pressure developed in the extruder barrel during the extrusion of fish muscle-rice flour blend. Higher barrel temperature also increases starch gelatinization and pressure inside the extruder, resulting in more expansion of the product. According to Choudhury and Gautum (1999), higher temperature at the die caused more flashing of water at the die, which resulted in higher overall expansion and reduced density. The results agree with the results from this study (Figure 19-B and C). Launary and Lisch (1983) suggested that the radial expansion was most dependent on the melt elasticity. The stored energy was released during expansion and increased radial expansion. Increased feed moisture during extrusion increases changes in the amylopectin molecular structure of the starch-based material and reduces melt elasticity which decreases the radial expansion ratio.

2.4.3 Shear Strength

The shear strength of the extrudates ranged from 187-374 N/cm^2 (Table 20). Although the R^2 value was high and lack of fit not significant ($p>0.05$). The positive regression coefficients of barrel temperature and feed moisture indicated that increased temperature and feed moisture might be associated with increased extrudate shear strength. In contrast, increased screw speed decreased the extrudate shear strength. The contour plot (Figure 20) shows the effect of screw speed and feed moisture on extrudate shear strength at barrel temperature of 135°C. Increased screw speed at given feed moisture at screw speed lower than 250 rpm incrased shear strength, whereas increased feed moisture at screw speed over 250 rpm decreased it.

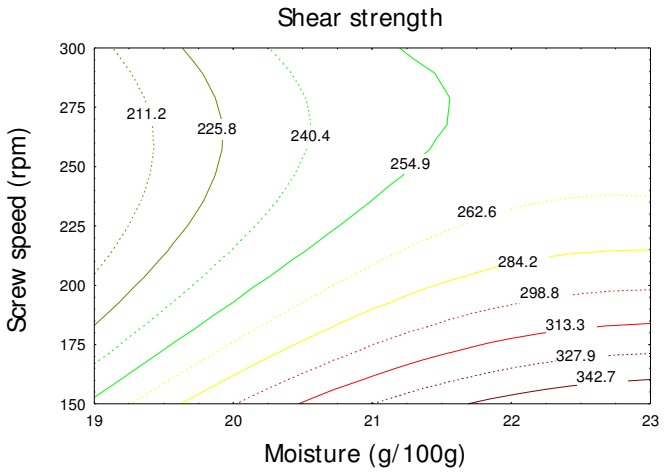


Figure 20 Contour plots of screw speed and feed moisture on shear strength (N/cm^2) of the extrudates at the barrel temperature of 135°C

Ding *et al.* (2006) reported similar results in extruded wheat-based expanded snack. They stated that incrased screw speed expected to lower the melt viscosity of the mix resulting in a less dense, softer extrudate. Increased feed moisture content leads to an increase in hardness.

2.5 Effects of Extrusion Conditions on Sensory Properties of the Extrudates

Changes in the physical and chemical properties of the extrudate ingredients influence the appearance, aroma, flavor and texture of the extruded products (Chen *et al*., 1991). In this study, the sensory properties were studied using hedonic rating score of 1-dislike extremely to 9-like extremely. Forty-five Asians, consisting of staff and students in the department of Food Science and Technology and members of Thai Students Association at the University of Georgia, Athens, GA. were recruited as panelists. Products were randomly served using a balanced incomplete block design (Cochran and Cox, 1950). Each panelist received five samples. Panelists were asked to evaluate the product attributes (appearance, color, aroma, flavor, texture and overall acceptability) using a nine-point hedonic rating score. The hedonic score ranges for appearance, color, aroma, flavor, texture and overall acceptability were from 4.90-6.63, 5.10-6.40, 4.30-6.38, 5.00-6.50, 5.00-6.89 and 5.00-6.50, respectively (Table 22).

Means of hedonic rating score sensory attributes were used to generate predictive regression models (Table 23). All predictive models shown high R^2 values, but there was model lack of fit for product overall acceptability and flavor acceptability. Additionally, none of the individual extrusion parameters were significant ($p>0.05$) in the models for appearance, aroma, flavor and texture. The linear and quadratic term of barrel temperature had significant effects ($p\leq0.05$) on the models for color and overall acceptability.

Table 22 Means of hedonic rating scores for sensory attributes and overall acceptability [a]

Experimental condition[a]			Mean ± S.D. of hedonic rating score[b]					
Temp	SS	M	Appearance	Color	Aroma	Flavor	Texture	Overall
125	225	23	5.4±1.58	5.4±1.50	5.2±1.40	5.7±1.49	5.8±1.81	5.2±1.62
	150	21	5.8±1.47	5.6±1.71	5.7±1.06	6.2±1.32	6.4±1.58	6.1±1.19
	300	21	6.6±0.92	6.1±1.55	6.4±0.74	6.5±1.19	6.4±1.41	6.5±1.41
	225	19	6.3±0.82	6.2±0.79	5.5±0.97	6.2±1.31	6.6±0.97	6.5±0.97
135	150	23	4.9±1.10	5.1±1.19	4.9±1.10	5.5±1.27	5.0±1.69	5.0±1.25
	225	**21**	**6.1±0.99**	**5.9±1.25**	**4.8±1.05**	**5.4±1.99**	**5.6±1.92**	**5.3±1.90**
	225	**21**	**5.9±0.99**	**5.3±1.25**	**4.3±1.05**	**5.3±1.42**	**6.5±1.26**	**5.3±1.42**
	225	**21**	**5.6±1.57**	**5.8±1.23**	**5.2±1.31**	**5.4±1.35**	**5.4±1.35**	**5.4±1.50**
	300	23	5.2±1.56	5.3±1.58	5.6±1.24	5.8±1.39	5.8±1.64	5.6±1.23
	150	19	6.0±0.87	5.4±1.51	4.8±1.92	5.0±1.94	6.9±1.26	5.6±1.42
	300	19	6.0±1.22	6.0±1.22	5.6±1.24	5.7±1.58	6.9±1.36	5.6±1.59
145	225	23	5.7±0.87	5.8±1.09	5.2±1.72	5.7±1.58	6.0±1.32	5.4±1.33
	150	21	5.9±1.13	6.0±1.31	5.4±1.50	5.3±1.93	6.5±1.06	6.1±1.25
	300	21	5.6±1.26	6.4±0.97	4.6±0.69	5.5±1.72	5.7±1.49	5.0±1.49
	225	19	5.7±0.95	5.8±1.48	5.3±1.34	6.0±1.33	6.9±0.73	6.1±0.87

[a] Temp = temperature (°C) of barrel zone 2, 3, and 4, SS = screw speed (rpm), and M = feed moisture (g/100g db)

[b] Mean ± SD of hedonic rating score from 15 panelists, each panelist tested 5 samples The score of 1=dislike extremely, 5=neither like of dislike, 9= like extremely.

Table 23 Coefficients of parameters in the regression models for sensory properties; appearance, color, aroma, flavor, texture and overall acceptabilty of extruded snack according to the independent variables of barrel temperature (X_1), screw speed (X_2) and feed moisture content (X_3)

Parameter	Parameter coefficients [a]					
	Appearance	Color	Aroma	Flavor	Texture	Overall acceptability
Intercept	33.039	70.333	95.984	103.028	82.062	114.653
Temperature (X_1)	– 0.618	**– 1.1320**	– 1.151	– 1.293	– 0.708	**– 1.432**
Screw speed (X_2)	0.045	0.0180	0.045	0.015	– 0.0009	0.039
Moisture (X_3)	0.159	0.9500	– 1.602	– 0.981	– 2.395	– 1.346
Temperature×Temperature (X_1^2)	0.002	**0.004**	0.004	0.004	0.002	**0.005**
Temperature×Screw speed (X_1X_2)	– 0.0004	– 0.00004	– 0.0004	– 0.0001	– 0.0003	– 0.0005
Screw speed×Screw speed (X_2^2)	– 0.00001	0.000004	0.00005	0.000008	0.00002	0.00002
Temperature×Moisture (X_1X_3)	0.011	0.009	0.003	0.002	– 0.001	0.008
Moisture×Screw speed (X_2X_3)	0.0005	– 0.0006	– 0.002	– 0.0007	0.001	0.0009
Moisture×Moisture (X_3^2)	– 0.069	– 0.058	– 0.030	– 0.019	0.047	– 0.002
R^2	0.89	0.86	0.67	0.72	0.72	0.81
Model lack of Fit	0.60	0.87	0.46	0.01	0.64	0.03

[a] Bold numbers are significant ($p \leq 0.05$) to the regression model

Contour plots generated from regression models showing trends of the effects of extrusion parameters on sensory attributes are presented in Figure 21-24. The following effects were noted:

1.) Increased feed moisture levels at any barrel temperature decreased the mean hedonic scored for appearance when the screw speed was constant (Figure 21).

2.) Increased barrel temperature decreased the mean hedonic score for appearance when screw speed was constant (Figure 21).

3.) The mean hedonic score for color (Figure 22-A), aroma (Figure 22-B) and flavor (Figure 23-A) increased as screw speed increased and feed moisture decreased at constant barrel temperature.

4.) The mean hedonic score for texture acceptability (Figure 23-B) increased as screw speed decreased and feed moisture increased.

5.) The patterns for overall acceptability were similar at the three temperatures. Figure 24 shows the effects of moisture and screw speed at 135°C. The major effect was a decrease in overall acceptability with increasing moisture.

In conclusion, the products with higher hedonic scores were produced at the extrusion conditions of medium to high barrel temperature, low screw speed, and a low feed moisture. The extrudates from these conditions had low bulk density and high expansion ratio, showing more puffing. Suknark *et al*. (1998) found that fried fish crackers and peanut crackers with low bulk density and higher expansion ratio were more acceptable to Asian and American consumers. Liu *et al*. (2000) studied texture profiles of extruded oat-corn products. The authors reported that increased feed moisture levels increased fracturability and hardness of the products. Increased moisture content or decreased screw speed decreased cohesiveness. Principal Component Analysis (PCA) confirmed that high initial feed moisture reduced product expansion by decreasing product temperature.

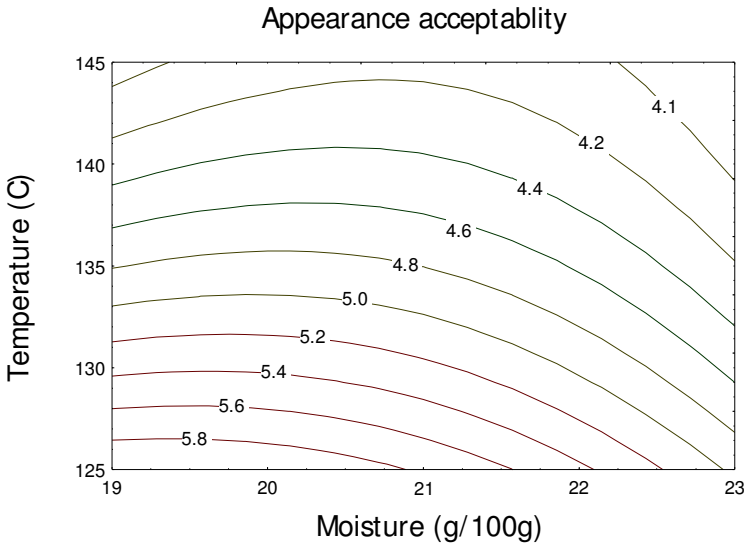
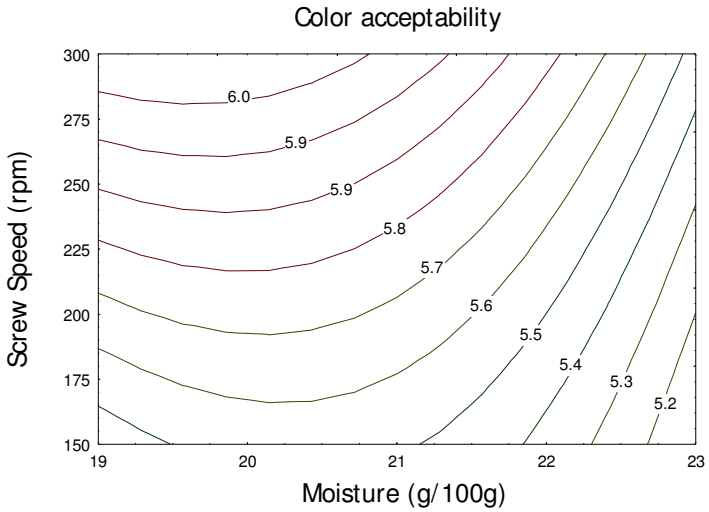
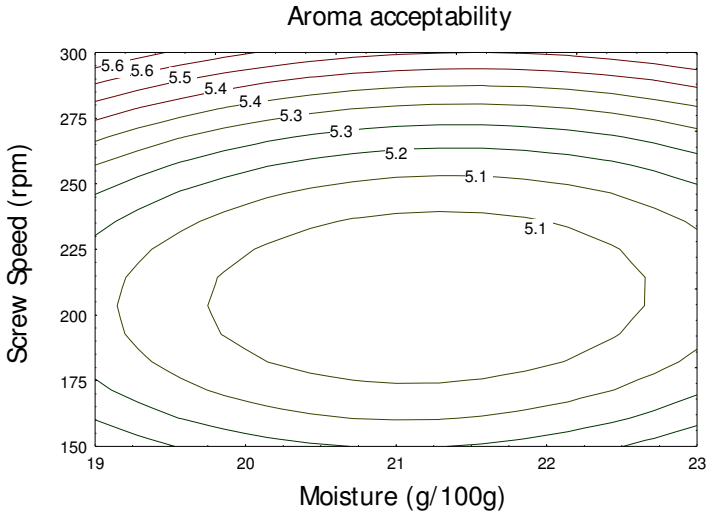


Figure 21 Contour plots of temperature and feed moisture on the appearance acceptability of the extrudate at the screw speed of 225 rpm

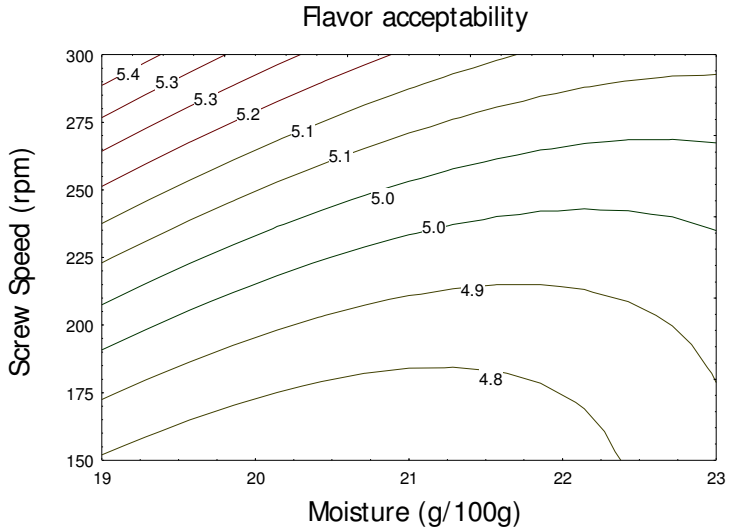


(A)

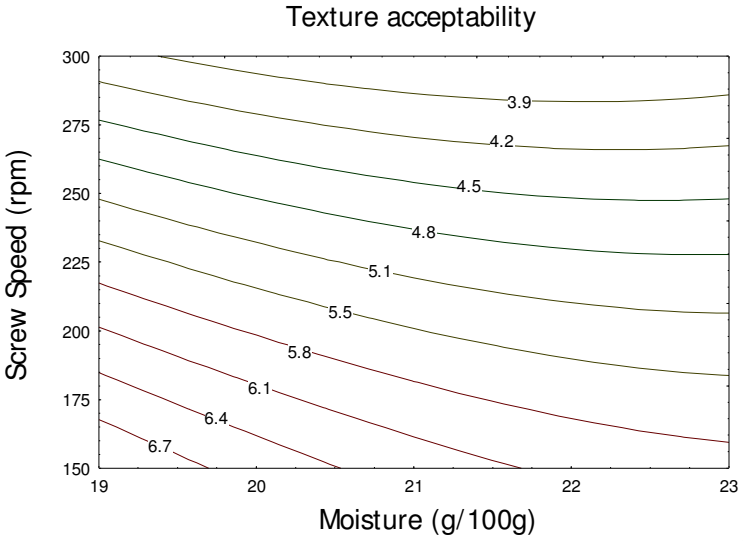


(B)

Figure 22 Contour plots of screw speed and feed moisture on the color acceptability (A) and aroma acceptability (B) of the extrudate at the barrel temperature of 135°C



(A)



(B)

Figure 23 Contour plots of screw speed and feed moisture on the flavor acceptability (A) and texture acceptability (B) of the extrudate at the barrel temperature of 135°C

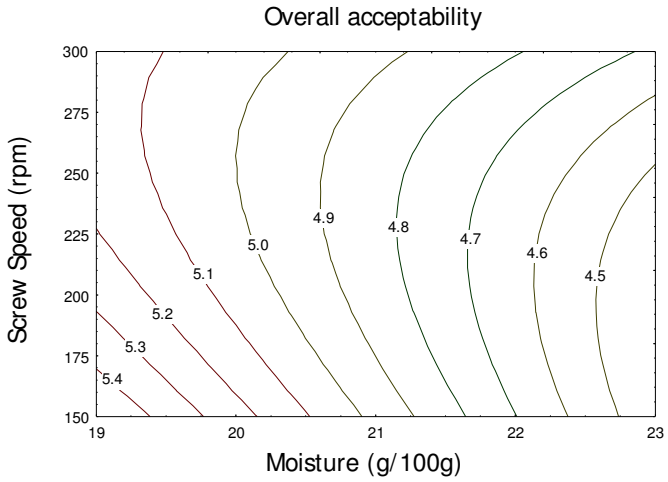


Figure 24 Contour plots of screw speed and feed moisture on the overall acceptability of the extrudate at the barrel temperature of 135°C

Barrel temperature, screw speed and moisture content determine not only the bulk density and expansion ratio but also the extrudate's internal structure, both of which influence the textural properties of the final product (Mezreb *et al*., 2003). Structure of the extrudates depends on the organization of cells in the extrudate and the cell size (Moore *et al*., 1990). In this study, consumers liked the texture of products that were extruded at low screw speed and low moisture content with relatively low bulk density and high expansion ratios. However, color, aroma and flavor of products that were produced at high screw speed and low feed moisture received higher acceptability scores. Liu *et al*. (2000) found that high bulk density were usually accompanied by some negative sensory attributes, such as roughness, irregular shape, and dry surface. Product expansion ratio was highly correlated with the cohesiveness and springiness and was associated with crispy texture and shinny surface.

Faller and Heymann (1996) investigated the effects of potato granule type (flake and flout), moisture (16%, 18% and 20%) and addition of oil (0%, 2% and 4%) on sensory attributes of extrudates. The products were extruded using co-rotating twin screw extruder. Generalized procrusted analysis (GPA) and principal component Analysis (PCA) were used to describe the relationship between selected sensory and physical variables. The analysis showed high feed moisture extrudates were high in hardness and chewiness. Whereas, low moisture ones were high in browness, burnt flavor and fracturability.

De Stefano *et al*. (2001) studied the effect of selected extrusion variables, die temperature (140-160°C) and screw speed (250-300rpm), on sensory attributes of corn, soybean and sorghum extrudates. The panelists preferred extrudate produced at 160°C die temperature and 300 rpm screw speed more than extrudate produced at 140°C. The product was described as having more roasted flavor and smoother texture than the product obtained at lower die temperature (140°C) and lower screw speed (250 rpm). Chang *et al*. (1998) developed an extruded snack using Jatobá (*Hymeaea stigonocapa* Mart) flour and cassava starch blend. The mixtures of Jatobá

flour and cassava starch were extruded at barrel temperatures of 125-175°C and moisture contents of 17-23%. Processing temperature significantly affected ($p \leq 0.05$) the appearance of the extrudate. Increased processing temperature up to the limit of 150°C increased hedonic score of the appearance but decreased at higher temperature.

In this study, the overall acceptability of the products extruded at feed moisture below 21 g/100g was more than 5.0 (5 = neither like or dislike). Relatively low overall acceptability might be because the products were prepared without addition of any seasoning or flavor. Some of the panelists commented that the products were plain in taste. The product might get higher acceptability scores after seasoning and flavor are added. The consumer survey conducted in Bangkok, Thailand (Section 1) showed the flavor that consumers prefer is BBQ. Therefore, this flavor will be added to the product for the consumer test in Thailand.

3. Optimization of the Extrusion Conditions of the Fish Snack

3.1 Optimum Extrusion Conditions

After the extrudates from 15 experimental conditions and raw material were analyzed for chemical, physical and sensory properties, predictive regression models and contour plots were generated. The extrusion conditions that provided high retention of EPA and DHA with acceptable sensory quality were selected as an optimum region.

Superimposition from these contour plots is illustrated in Figure 25. The area above the constraint values, EPA+DHA content (>810 mg/100g) and overall acceptability (hedonic score > 5), was considered to be the optimum region. The optimum region of the extrusion conditions for the extruded fish snack were at the screw speed of 240-300 rpm and moisture content of 19.0-19.5 g/100g (db). These extrudates showed a lower bulk density, higher expansion, less hardness higher sensory quality.

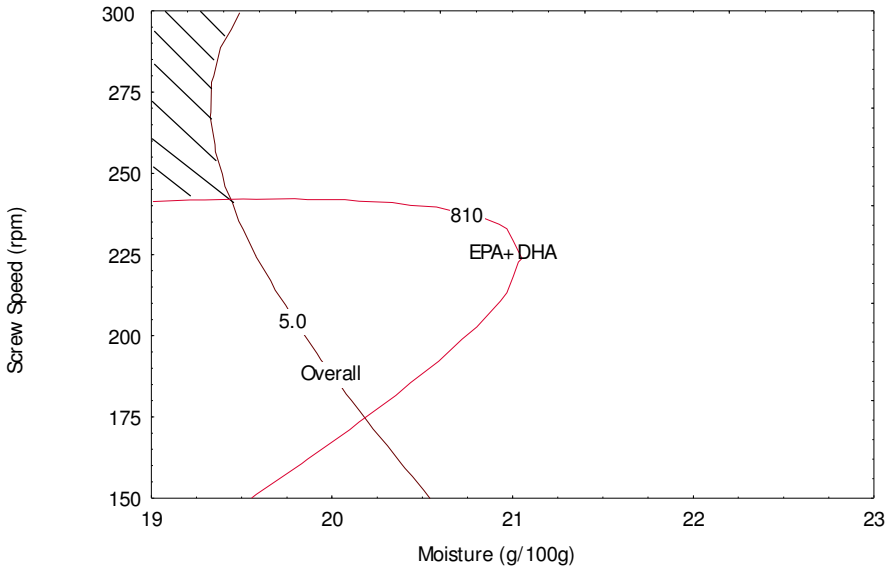


Figure 25 Optimum screw speeds and feed moistures for extruding extruded snack supplemented with fish protein and n-3 fatty acids at the barrel temperature of 135°C

The extrusion conditions that provided EPA+DHA content above 810 mg/100g (~90%) and sensory acceptability score above 5 were selected to determine the optimum region for extrusion. The contour plot generated from the predictive regression model of vitamin E contents was presented as a function of barrel temperature and feed moisture. Therefore, it could not be superimposed upon other contour plots that were presented as functions of screw speed and feed moisture. Since hedonic scores of all sensory characteristics were higher than 5 (5 = neither like of dislike) with the exception of the score for appearance (Figure 22), overall acceptability was chosen as the criterion for sensory properties.

3.2 Predictive Regression Model Verification

To study the reliability of the predictive regression model, an independent model verification study was performed. Three extrusion conditions were selected from the optimum region, and two extrusion conditions were selected from the area outside the optimum region (Table 24). The observed values were compared with those predicted by the regression models. Results are presented in Table 25 and 26. The predicted values from the model for EPA were lower than the observed value except at 135°C, 250 rpm and 19% moisture. The predicted values of DHA and EPA+DHA were higher than the observed value. The highest %error was found at 145°C, 150 rpm and 23% moisture. Percent error of predicted value of EPA, DHA and EPA+DHA ranges from 0.0-25.0. The highest %error was found for the DHA value difference at 145°C, 150 rpm and 23% moisture. Percent error of predicted value of total tocopherols and tocotrienols ranges from 0.3 to –12.9%. The highest % error was found at 125°C, 300 rpm and 19% moisture (Table 25).

The predicted values under estimated (predicted) bulk density from 6.7 to 20.7%. In contrast, expansion ratio was over estimated from 2.9 to 18.9%. Percent error of predicted hedonic scores for overall acceptability ranged from 2.5 to –30.4 (Table 26). While the ranges of percent error for the specific analytes vary by the processing parameters, they indicate that the models accurately describe the effects of the extrusion variables on product characteristics.

Table 24 Extrusion conditions for model verification.

Treatment	Extrusion conditions		
	Temperature (°C)	Screw speed (rpm)	Moisture content (g/100g)
1	125	300	19
2	135	250	19
3	135	300	19
4[a]	135	250	22
5[a]	145	150	23

[a] extrusion conditions outside the optimization area

Table 25 Observed and predicted values of EPA, DHA, EPA+DHA and total tocopherolsand tocotrienols

Extrusion condition			EPA (mg/100g)			DHA (mg/100g)			EPA+DHA (mg/100g)			Total tocopherols and tocotrienols (mg/100g)		
Temperature (°C)	Screw speed (rpm)	Moisture (g/100g)	Observed value	Predicted value	% error	Observed value	Predicted value	% error	Observed value	Predicted value	% error	Observed value	Predicted value	% error
125	300	19	340 ±15	324	-4.9	552	846 ±20	8.3	846 ±20	884	4.3	0.32 ±0.022	0.30	-12.9
135	250	19	345 ±7	315	-8.6	510	855 ±12	0.0	855 ±12	830	-3.0	0.35 ±0.033	0.29	-2.7
135	300	19	349 ±10	350	0.3	547	872 ±31	4.4	872 ±31	905	3.6	0.31 ±0.035	0.28	-0.7
135	250	22	347 ±27	301	-15.3	525	857 ±67	2.9	857 ±67	831	-3.1	0.41 ±0.033	0.35	0.3
145	150	23	314 ±3	306	-2.6	603	767 ±6	25.0	767 ±6	910	15.7	0.42 ±0.064	0.37	8.4

Table 26 Observed and predicted values of product density, expansion ratio and overall acceptability

Extrusion condition			Product density (g/cm3)			Expansion ratio			Overall acceptability		
Temperature (°C)	Screw speed (rpm)	Moisture (g/100g)	Observed value	Predicted value	% error	Observed value	Predicted value	% error	Observed value	Predicted value	% error
125	300	19	0.32 ±0.022	0.30	-6.7	2.40 ±0.101	2.55	5.9	6.2 ±0.83	6.4	2.5
135	250	19	0.35 ±0.033	0.29	-20.7	2.31 ±0.098	2.38	2.9	6.1 ±1.51	5.1	-20.1
135	300	19	0.31 ±0.035	0.28	-10.7	2.37 ±0.080	2.43	2.5	6.6 ±1.36	5.1	-30.4
135	250	22	0.41 ±0.033	0.35	-17.1	2.14 ±0.068	2.29	6.6	5.3 ±1.27	4.7	-12.5
145	150	23	0.42 ±0.064	0.37	-13.5	2.14 ±0.085	2.64	18.9	4.5 ±1.27	5.3	15.6

CONCLUSION

Responses pertaining to perception and attitude from the consumer survey study conducted in Bangkok, Thailand, showed that products containing fish and fish oil were acceptable by the recruited consumers. Most consumers were aware of the beneficial heath effects of fish and/ or fish oil, thus, there is a potential to develop a nutritious snack that would be accepted by Thai consumers.

A mixture of rice flour, fish powder, fish oil and mixed tocopherol containing 900 mg of n-3 fatty acids per 100 g db was extruded at barrel temperatures of 125-145°C, screw speeds of 150-300 rpm and feed moistures of 19-23 g/100g db. Retentions of n-3 fatty acids (EPA+DHA) and vitamin E (total tocopherols and tocotrienols) in extruded products were more than 75% and 54%, respectively. Extrudates with higher hedonic scores were produced at the extrusion conditions of medium to high barrel temperature (135-145°C), high screw speed (300 rpm), and low feed moisture (19 g/100g db). The extrudates from these conditions had low product density and high expansion ratio indicated more puffing.

Contour plots generated from redictive regression models for EPA+DHA retention and consumer acceptality were use to identify the optimum extrusion condition. The optimum region was characterized by EPA+DHA retention >90% (810mg/100g) and hedonic scores for overall acceptability >5 (5=neither like or dislike). This region was located at a barrel temperature of 135°C, screw speed of 240-300 rpm and moisture content of 19.0-19.5 g/100g (db). The products at these extrusion conditions showed lower product density, higher radial expansion and lower shear strength than at other conditions. Independent verification extrusion experiments were performed at three extrusion conditions in the optimum region and two outside the optimum region. The resulting extrudates were analyzed for EPA, DHA, total tocopherols, product density, expansion ratio and overall acceptability. The observed values were compared to predicted values to study the reliability of the

predictive regression. The difference between observed and predicted values ranged from 0.0 to 30.4%.

The effects of extrusion variables are complicated. They not only affect extrudate properties, but also influence each other. Therefore, explanation of the effect of individual extrusion parameter could not be done without considering other parameters. In this study, screw speed and feed moisture affected chemical, physical and sensory properties of extrudates more than those of temperature. Increased screw speed or increased feed moisture increased retention of EPA, DHA, and thus, EPA+DHA. The highest retention of vitamin E was found at high screw speed and high feed moisture. The lowest retentions of vitamin E were found where the retentions of EPA and DHA were highest. This might relate to the antioxidant functions of tocopherols and tocotrienols, showing the degradation of vitamin E and the protection of the n-3 fatty acids. The highest retentions of n-3 fatty acids were found at higher screw speed and low feed moisture and at lower screw speed at high feed moisture. The latter extrusion conditions might not produce the best products from a consumer standpoint. Addition of 0.005% (500 ppm) of mixed tocopherols was sufficient to protect the n-3 fatty acids. Higher levels of addition might produce pro-oxidant activity.

It is likely that alternative ingredients for producing rice flour, fish power and fish oil extrusion snack could also be used. For example, the underutilized and inexpensive fish can be made to fish powder to use as an ingredient to make this more value added product. However, it should be considered that changes in ingredient compositions or extrusion conditions might lead different results. The predictive regression models from this study cannot be used beyond the experimental range. However, they can be guidelines to start a new extrusion experiment.

LITERATURE CITED

Adam, O., C. Beringer, T. Kless, C. Lemmen, A. Adam, M. Wiesman, P. Adam, R. Klimmek and W. Forth. 2003. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. **Rheumatol. Intl.** 23: 27-36.

Ainsworth, P., S. Ibanoglu and G.D. Hayes. 1997. Influence of process variables on residence time distribution and flow patterns of tarhana in a twin-screw extruder. **J. Food Eng.** 32: 101-108.

Akdogan, H. 1996. Pressure, torque, and energy responses of a twin screw extruder at high moisture contents. **Food Res. Int.** 29: 423-429.

Amer, M.A., P.B. Kupranycz and B.E. Baker. 1985. Physical and chemical characteristics of butter fat fractions obtained by crystallization from malten fat. **JAOCS.** 62: 1551-1557.

Anon. 2002. Opting for omegas. **Prepared Foods** 171(6): 44.

AOAC. 1995. **Official Methods of Analysis of the Association of Official Analytical Chemists International.** 16th ed. The Association of Official Analytical Chemists, Inc., Arlington, VA.

Areas, J.A.G. 1992. Extrusion of food protein. **Crit. Rev. Food Sci. Nutr.** 32(4): 365-392.

Arkhipenko, Y.V. and T.G. Sazontova. 1995. Review: mechanisms of the cardioprotective effect of a diet enriched with ω-3 polyunsaturated fatty acids. **Pathophysiol** 2: 131-140.

Augustin, M.A. and L. Sanguansri. 2003. Polyunsaturated fatty acids: delivery, innovation and incorporation into foods. **Food Aust.** 55(7): 294-296.

Augustsson, K., D.S. Michaud, E.B. Rimm, M.F. Leitzmann, M.J. Stampfer, W.C. Willett and E. Giovannucci. 2003. A prospective study of intake of fish and marine fatty acids and prostate cancer. **Cancer Epidermiol. Biomarkers Prev.** 12(1): 64-67.

Baik, M-Y., E.L. Suhendro, W.W. Newar, D.J. McClements, E.A. Decker and P. Chinachoti. 2004. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. **JAOCS.** 81(4): 355-360.

Ball, G.F.M. 1988. **Fat Soluble Vitamin Assays in Food Analysis**. Elsevier Applied Science, New York.

Bender, D.A. 2003. Vitamin E: tocopherols and tocotrienols, pp. 109-130. *In* D.E. Bender, ed. **Nutritional Biochemistry of the Vitamins.** 2nd ed. Cambridge University Press, Cambridge.

Bindzus, W., S.J. Living, H. Gloria-Hernandez, G. Fayard, B. van Lengerich and F. Meuser. 2002. Glass transition of extruded whear corn and rice starch. **Starch** 54: 394-400.

Box, G.E.P. and D.W. Behnken. 1960. Some new three level designs for the study of quantitative variables. **Technometrics** 2(4): 455-475.

Burton, G.W., A. Joyce and K.U. Ingold. 1983. Is vitamin E the only lipid-soluble chain breaking antioxidant in human blood plasma and erythrocyte membrane? **Archives of Biochemistry and Biopolymer**s 21: 281-290.

_____ and K.U. Ingold. 1989. Vitamin E as in vitro and vivo antioxidant. **Ann. N.Y. Acad. Sci.** 570: 7-22.

Caicoya, M. 2002. Fish consumption and stroke: a community case-study in Asturius, Spain. **Neuroepidemiology** 21(3): 105-106.

Camire, M.E. 1998. Chemical changes during extrusion cooking, pp. 109-121. *In* **Process-Induced Chemical Changes in Food.** F. Shahidi, C-T. Ho and N.V. Chuyen, eds. Plenum Press, New York.

Camire, M.E. 2000. Chemical and nutritional changes in food during extrusion, pp. 127-147. *In* M.N. Riaz, ed. **Extruders in Food Applications.** Technomic Publising Co., Inc., Pensylvania.

Chang, Y.K., M.R. Silva, L.C. Gutkoski, L. Sebio and M.A.A.P. Da Silva. 1998. Development of extruded snacks using Jatoba (*Hymenaea sitonocarpa* Mart) flout and cassava starch blends. **J. Sci. Food Agric.** 78: 59-66.

Charoenphol, C., A. Nitithamyong and V. Chavisit. 1992. Fish Crispy: A high protein extruded snack, pp. 24-25. *In* **Asia Pacific Food Technology Symposium Conference Proceeding.** Kuala Lumper, Malaysia.

Cheftel, J.C. 1986. Nutrition effects of extrusion cooking. **Food Chem.** 20: 263-283.

Chen, C-M. and A-I. Yeh. 2001. Effect of amylose content on expansion of extruded rice pellet. **Cereal Chem.** 78(3): 261-266.

Chen, J., F.L. Serafin, R.N. Pandya and H. Duan. 1991. Effects of extrusion conditions on sensory properties of corn meal extrudates. **J. Food Sci.** 56(1): 84-89.

Choudhury, G.S. and A. Gautam. 1998. Comparative study of mixing elements during twin-screw extrusion of rice flour. **Food Res. Int.** 31(1): 7-17.

Choudhury, G.S. and A. Gautam. 1999. Screw configuration effects on macroscopic characteristics of extrudates produced by twin-screw extrusion of rice flour. **J. Food Sci.** 64(3): 479-487.

_____ and _____. 1999. Screw configuration effects on macroscopic characteristics of extrudates produced by twin-screw extrusion of rice flour. **J. Food Sci.** 64(3): 479-487. *Cited* W.A. Yacu. 1995. Thermoplastic and food extrusion general introduction, pp. 1-37. *In* **Food Extrusion Technology (Short course).** The Center for Professional Advancement, East Brunswick, New Jersy.

_____ and _____. 2003. Effects of hydrolysed fish muscle on intermediate process variables during twin-screw extrusion of rice flour. **Lebensm.-Wiss. u.-Technol.** 36: 667-678.

Chow, C.K. 2001. Vitamin E, pp. 165-198. *In* R.B. Rucker, J.W. Suttie, D.B. McCormick and L.J. Machlin, eds. **Handbook of Vitamin.** 3rd ed. Marcel Dekker Inc., New York.

Cleland, L.G., M.J. James and S.M. Proudman. 2003. The role of fish oils in the treatment of rheumatoid arthritis. **Drugs** 63(9): 845-853.

Cochran, W.G. and G.M. Cox. 1950. Experimental Design. John Wiley & Sons, New York.

Connor, W.E. 2000. Importance of n-3 fatty acids in health and disease. **Am. J. Clin. Nutr.** 71(suppl): 171S-175S.

Davis, E.A., J. Grider and J. Gordon. 1986. Microstructural evaluation of model starch system containing different types of oils. **Cereal Chem.** 63: 427-430.

De Baun, R.M. 1959. Response surface designs for three factors at three levels. **Technometrics** 1(1): 1-8.

De Caterina, D. and R. Madonna and R. Zucchi. 2003. Antiarrhythmic effects of omega-3 fatty acids: from epidemiology to bedside. **Am. Heart J.** 146(3): 420-430.

De Stefano, G., P. Piacquadio and V. Scencalepore. 2001. Effect of selected properties processing variables on sensory attributes of extrudates. **Itl. J. Food Sci.** 2(13): 29-232.

Desrumaux, A., J.M. Bouvier and J. Burri. 1999. Effect of free fatty acids addition on corn grits extrusion cooking. **Cereal Chem.** 76(5): 699-704.

Dickinson, A. 2002. Benefit of long chain omega-3 fatty acids (EPA, DHA): help protect against heart disease. *In* **The Benefits of Nutritional Supplements**. Council for Responsible Nutrition (CRN), Washington D.C.

Din, J.N., D.E. Newby and A.D. Flapan. 2004. Omega-3 fatty acids and cardiovascular disease-fishing for a natural treatment. **BMJ.** 328: 30-35.

Ding, Q-B., P. Ainsworth, A. Plunkett and H. Marson. 2006. The effects of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. **J. Food Eng.** 73: 142-148.

Druizer, L.M., O.H. Campanella and G.R.G. Barnes. 1998. Sensory, Instrumental and acoustic characteristic of extruded snack food products. **J. Food Texture Stud.** 29(4): 397-411.

Eitenmiller, R.R. and J. Lee. 2004. **Vitamin E: Food Chemistry, Composition and Analysis**. Marcel Dekker, Inc., NewYork.

Ergas, D., E. Eilat, S. Mendlovic and Z.M. Sthoeger. 2002. n-3 Fatty acids and the immune system in autoimmunity. **IMAJ.** 4:34-38.

Erkkilä, A.J., S. Lehto, K. Pyörälä and M.I.J. Uusitupa. 2003. n-3 Fatty acids and 5-y risks of death and cardiovascular disease event in patients with coronary artery disease. **Am J. Clin. Nutr.** 78:65-71.

Falcone, R.G. and E.D. Phillips. 1988. Effects of feed composition, feed moisture, and barrel temperature on the physical properties of snack-like products prepared from cowpea and sorghum flours by extrusion. **J. Food Sci.** 53(5): 1464-1469.

Faller, J.Y. and H. Heymann. 1996. Sensory and physical properties of extruded potato puffs. **J. Sens. Stud.** 11: 227-245.

Fan, J., J.R. Mitchell and J.M.V. Blanshard. 1994. A computer simulation of the dynamics of bubble growth and shrinkage during extrudate expansion. **J. Food Eng.** 23: 337-356.

Fanta, G.F. and S.K. Eskins. 1995. Stable starch-lipid compositions prepared by steam jet cooking. **Carbohydr. Polym.** 28: 171-175.

FDA/ CFSAN. 2000. **Letter regarding dietary supplement health claim for omega-3 fatty acids and coronary heart disease (Docket No. 91N-0103)**. U.S. Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Nutritional Products, Labeling, and Dietary Supplements. Washington, D.C.

_____. 2002. **Letter regarding dietary supplement health claim for omega-3 fatty acids and coronary heart disease (Docket No. 91N-0103).** U.S. Food and Drug Administration. Center for Food Safety and Applied Nutrition. Office of Nutritional Products, Labeling, and Dietary Supplements. Washington, D.C.

Fichtali, J. and van de Voort, F.R. 1989. Fundamental and practical aspects of twin screw extrusion. **Cereal Food World** 34(11): 921-929.

Floros, J.D. and M.S. Chinnan. 1987. Computer graphic-assisted optimization for product and process development. **Food Tech.** 72(2): 74-78, 84.

Food and Drug Administration (FDA). 2004. An announces qualified health claims for omega-3 fatty acids. **FDA News**, September 8, 2004.

Frame, N.D. 1994. **The Technology of Extrusion Cooking**. Blackie Academic & Professional, London.

Geise, J. 1996. Antioxidants: tools for preventing lipid oxidation. **Food Tech.** 50: 73-80.

Giri, S.K. and S. Bandyopadhyay. 2000. Effect of extrusion variables on extrudate characteristics of fish muscle-rice flour blend in a single-screw extruder. **J. Food Process Preserv.** 24:177-190.

GISSI Prevenzione Investigator. 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. **LANCET** 354: 447-455.

Gogoi, B.K. and K.L. Yam. 1994. Relationships between residence time and process variables in a co-rotating twin screw extruder. **J. Food Eng.** 21: 177-196.

_____, A.J. Oswalt and G.S. Choudhury. 1996a. Effects of location and spacing of reverse screw and kneading element combination during twin-screw extrusion of starchy and proteinaceous blends. **Food Res. Int.** 29(5-6): 505-512.

Gogoi, B.K., A.J. Oswalt and G.S. Choudhury. 1996b. Reverse screw element(s) and feed composition effects during twin-screw extrusion of rice flour and fish muscle blends. **J. Food Sci.** 61(3): 509-595.

Guha M., S. Z. Ali and S. Bhattacharya. 1997. Twin-screw extrusion of rice flour without a die: effect of barrel temperature and screw speed on extrusion and extrudate characteristics. **J. Food Eng.** 32: 251-267.

_____, _____ and _____. 2003. Screening of variables for extrusion of rice flour employing a Plackette-Burman design. **J. Food Eng.** 57: 135-144.

Hamilton, R.J., C. Kalu, E. Prisk, F.B. Padley and H. Pierce. 1997. Chemistry of free radical in lipids. **Food Chem.** 60(2): 193-199.

_____, _____, G.P. McNeil, F.B. Padley and J.H. Pierce. 1998. Effects of tocopherols, ascoryl palmitate and lecithin on autoxidation of fish oil. **JAOCS.** 75(7): 813-822.

Harper, J.M. 1992. A comparative analysis of single- and twin-screw extruders, pp. 139-148. *In* J.L. Kokini, C. Ho and M. Karwe, eds. **Food Extrusion Science and Technology**. Marcel Dekker Inc., New York.

Hashimoto, J.M., E.H. Nabeshima, H.S. Cintra, A.R.G. Dias, F.M. Bustos and Y.K. Chang. 2002. Effect of processing conditions on some functional characteristics of extrusion-cooked cassava starch/wheat gluten blend. **J. Sci. Food Agric.** 82: 924-930.

He, K., E.B. Rimm, A. Merchant, B.A. Rosner, M.J. Stampfer, W.C. Willet and A.A. Ascherio. 2002. Fish consumption and risk of stroke in men. **JAMA.** 288(24): 3130-3136.

Horwitt, M.K. 2001. Critique of the requirement for vitamin E. **Am. J. Clin. Nutr.** 73: 1003-1005.

Hu, F.B., L. Bronner, W.C. Willett, M.J. Stampfer, K.M. Rexrode, C.M. Albert, D. Hunter and J.E. Manson. 2002. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. **JAMA.** 287(14): 1815-1821.

Hu, R. 1999. Food Product Designs: a Computer-Aided Statistical Approach. Technomic Publishing Co. Ltd., Pensylvania.

Ilo, S., U. Tomschik, E. Berghofer and N. Mundigler. 1996. The effects of extrusion operating conditions on the apparent viscosity and the properties of extrudates in twin-screw extrusion of maize grits. **Lebensm.-Wiss. u.-Technol.** 29: 593-598.

_____, Y. Liu and E. berghofer. 1999. Extrusion cooking of rice flour and amaranth blends. **Lebensm.-Wiss. u.-Technol.** 32: 79-88.

Institute of Medicine (IOM). 2000. Vitamin E, pp. 186-283. *In* **Dietary Reference Intake.** The National Academics Press, Washington, D.C.

_____. 2002. Dietary fats: Total fat and fatty acids, pp. 8-1-1-97. Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Part 1. Institute of Medicine of the National Academics. The National Academics Press, Washington, D.C.

Iso, H., K.M. Rexrode, M.J. Stamfer, J.E. Manson, G.A. Colditz, F.E. Speizer, C.H. Hennekens and W.C. Willett. 2001. Intake of fish and omega-3 fatty acids and risk of stroke in women. **JAMA.** 285(3): 304-312.

Iwe, M.O., D.J. van Zuilichem and P.O. Ngoddy. 2001. Extrusion cooking of blends of soy flour and sweet potato flour on specific mechanical energy (SME), extrudate temperature and torque. **J. Food Process Preserv.** 25: 251-266.

Jager, T., D.J. van Zuilichem, W. Stolp and K. vant Riet. 1991. Residence time distributions in extrusion cooking. Part VI: viscous dissipation in a drossel element in feed zone of a counter-rotating twin-screw extruder. **J. Food Eng.** 12: 67-82.

Jean, I.J., R. Work, M.E. Camire, J. Briggs, A.H. Barnett and A.A. Bushway. 1996. Selected properties of extruded potato and chicken meat. **J. Food Sci.** 61(4): 783-789.

Kaitaranta, J.K. 1992. Control of lipid oxidation in fish oil with various antioxidative compounds. **JAOCS.** 69(8): 810-813.

Kamal-Eldin, A. and L-Å. Appelqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. **Lipids** 31(7): 671-701.

_____, and N.V. Yanishlieva. 2002. n-3 Fatty acids for human nutrition: stability consideration. **Eur. J. Lipid Sci. Tech.** 104: 825-836.

Kaur, K and N. Singh. 2000. Amylose-lipid complex formation during cooking of rice flour. **Food Chem.** 71: 511-517.

Killiet, U. 1994. Vitamin retention in extrusion cooking. **Food Chem.** 49:149-155.

Kokini, J.L., C-T. Ho and M.V. Karwe. 1992. **Food extrusion Science and Technology.** Marcel Dekker Inc., New York.

Köksel, H., G-H. Ryu, A. Başman, H. Demiralp and P.K.W. Ng. 2004. Effects of extrusion variables on the properties of waxy hulless barley extrudates. **Narung** 48(1): 19-24.

Kolanowski, W., F. Swiderski and S. Berger. 1999. Possibilities of fish oil application for food products enrichment with n-3 PUFA. **Int. J. Food Sci Nutr.** 50: 39-49.

Kris-Etherton, P.M., D.S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R.L. Hargrove, G. Zhao and T.D. Etherton. 2000. Polyunsaturated fatty acids in the food chain in the United States. **Am. J. Clin. Nutr.** 71(suppl): 179s-188s.

_____, W.S. Harris and L.J. Appel. 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Circulation** 106: 2747-2757.

_____, _____and _____. 2003. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Arterioscler. Thromb. Vasc. Biol.** 23: e20-e31.

Kulås, E. and R.G. Ackman. 2001a. Properties of α-,γ- and δ-tocopherol in purified fish oil triacyl glycerols. **JAOCS.** 78(4): 361-367.

_____ and _____. 2001b. Different tocopherols and the relationship between two methods for determination of primary oxidation products in fish oil. **J. Agric. Food Chem.** 49: 1724-1729.

_____ and _____. 2001c. Protection of α-tocopherol in nonpurified and purified fish oil. **JAOAC.** 78(2): 197-203.

_____, E. Olsen and R.G. Ackman. 2002. Effect of α-, γ- and δ-tocopherol on the distribution of volatile secondary oxidation product in fish oil. **Eur. J. Lipid Sci. Tech.** 104: 520-529.

Larsson, S.C., M. Kumlin, M. Ingelman-Sundburg and A. Wolk. 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanism. **Am. J. Clin. Nutr.** 79: 935-945.

Launay, B. and J.M. Lisch. 1983. Twin-screw extrusion cooking of starches: flow behavior of starch pastes, expansion, and mechanical properties of extrudates. **J. Food Eng.** 2: 259-280.

Lawton, J.W., A.B. Davis and K.C. Behnke. 1985. High-temperature, short-time extrusion of wheat gluten and bran-like fraction. **Cereal Chem.** 62(4): 267-271.

Lee, S.Y. and K.L. McCarthy. 1996. Effect of screw configuration and speed on RTD and expansion of rice flour extrudate. **J. Food Eng.** 19: 153-170.

Lefevre, M. P.M. Kris-Etherton, G. Zhao and R.P. Tracy. 2004. Dietary fatty acids, hemostasis, and cardiovascular disease risk. **J Am. Diet. Assoc.** 104: 410-419.

Levenspeil, O. 1972. Residence time determination, pp. 253-278. *In* **Chemical Reaction Engineering.** 2nd ed. John Wiley and Sons Inc., New York,

Liu, Y, F. Hsies, H. Heymann ad H.E. Huff. 2000. Effect of process conditions on the physical and sensory properties of extruded oat-corn puff. **J. Food Sci.** 65(7): 1253-1259.

Lu, Q., F. Hsieh, S.J. Mulvaney, J. Tan and H.E. Huff. 1992. Dynamic analysis of process variables for twin-screw extruder. **Lebensm.-Wiss. u.-Technol.** 25(3): 261-269.

Machlin, L.J. 1984. Vitamin E, pp.99-144. *In* L.J. Machlin, ed. **Handbook of Vitamins: nutritional, biochemical and clinical aspect.** Marcel Dekker Inc., New York.

Mayer, R.H. and D.C. Montgomery. 1995. **Response Surface Methodology: Process and product optimization using designed experiments.** Wiley, New York. 700p.

Mayer, R.H., D.C. Montgomery, C.M. Borror and S.M. Kowalski. 2004. Response surface methodology: a retrospective and literature survey. **J. Quality Tech.** 36(1): 53-77.

Meuser, F and B. van Lengerich. 1984. System analytical model for the extrusion of Starches, pp. 175-179. *In* P. Zeuthen, J.C. Cheftel, C.Eriksson, M. Jul, H. Leniger, P. Linko, G. Varela and G. Vos, eds. **Thermal processing and quality of foods**. Elsevier Applied Sci. Publ., London.

Mezreb, K., A. Gaullieux, R. Ralainirina and M. Queneudec. 2003. Application of image analysis to measure screw speed influence on physical properties of corn and wheat extrudates. **J. Food Eng.** 57: 145-152.

Mohamed, S. 1990. Factors affecting extrusion characteristics of expanded starch-based products. **J. Food Process Preserv.** 14: 437-452.

Montgomery, D.C. 1984. **Design and Analysis of Experiment** 3rd ed. John Willey and Sons, Inc., New York. pp. 546-548.

Moore, G. 1994. Snack food extrusion, pp. 110-143. *In* N.D. Frame, ed. **The Technology of Extrusion Cooking.** Blackie Academic & Professional. London.

Nawar, W.W. 1996. Lipids, pp. 225-320. *In* O.R. Fennema, ed. **Food Chemistry**. 3rd ed. Marcel Dekker, Inc., New York.

Nettleton, J. 1995. **Omega-3 Fatty Acids and Health.** Chapman & Hall, New York.

Norrish, A.E., C.M. Skeaff, G.L.B. Arribas, S.J. Sharpe and R.T. Jackson. 1999. Prostate cancer risk and consumption of fish oils: dietary biomarker-based case-control study. **Brit. J. Cancer.** 81(7): 1238-1242.

Office of the Federal Register. 2003. **National Archives and Records.** Code of Federal Regulation. 9 CFR 424.21, 21 CFR 104.100, 21 CFR 181.1890, 21 CFR 182.3890. 21 CFR 182.8890. Administration. U.S. Government Printing Office, Washington, D.C.

Őzer, E.A., Ş. İbanoğlu, P. Ainsworth and C. Yağmur. 2004. Expansion characteristics of a nutritious extruded snack food using response surface methodology. **Eur. Food Res. Tech.** 218: 474-479.

Padmanabhan, M and M. Bhattacharya. 1989. Extrudate expansion during extrusion cooking of foods. **Cereal Food World** 34: 945-949.

Pan, B.S., M.S. Kong and H.H. Chen. 1992. Twin-screw extrusion for expanded rice products: processing parameters and formulation of extrudate properties, pp. 697-709.. *In* J.L. Kokini, C.-T. Ho and M.V. Karwe, eds. **Food Extrusion Science and Technology.** Marcel Dekker, Inc., New York.

Pan, Z., S. Zhang and J. Jane. 1998. Effect of extrusion variables and chemicals on the properties of starch-based binders and processing conditions. **Cereal Chem.** 75(4): 541-546.

Park, Y., S.D. Kelleher, D.J. McClements and E.A. Decker. 2004. Incorporation and stabilization of omega-3 fatty acids in surimi made from cod, *Gadus morhua*. **J. Agric. Food Chem.** 52: 597-601.

Peng, J., H.E. Huff and F. Hsieh. 1994. An RTD Determination method for extrusion cooking. **J. Food Process Preserv.** 18: 263-277.

Pokorny, J. 1987. Major factors affecting the autoxidation of lipids, pp. 141-206. *In* H.W.S. Chan, ed. **Autoxidation of Unsaturated Lipids.** Academic Press, London.

Ranhotra, G.S., J.A. Gelroth and E.M. Okot-Kotber. 2000. Stability and dietary contribution of vitamin E added to bread. **Cereal Chem.** 77(2): 159-162.

Riaz, M.N. 2000. Introduction to Extruders and their principles, pp 1-23. *In* M.N. Riaz, ed. **Extruder in Food Applications.** Technomic Publishing Company, Inc., Pensylvania

Rokey, G.J. 2000. Single screw extruders, pp. 25-49. *In* M.N. Riaz, ed**. Extruders in Food Applications.** Technomic publishing Co. Inc., Lancaster and Basel.

Rossen, J.L. and R.C. Miller. 1973. Food Extrusion. **Food Tech.** 27(8): 46-53.

Rupérez, F.J., D. Martin, E. Herrera and C. Barbas. 2001. Chromatographic analysis of α-tocopherol and related compounds in various matrices. **J. Chromatogr. A.** 935: 45-69.

Ryu, G.H. and P.K.W. Ng. 2001. Effects of selected process parameters on expansion and mechanical properties of wheat flour and whole cornmeal extrudates. **Starch** 53: 147-154.

Sang, W. and Z.T. Jin. 2004. Lipid oxidation of fish liver oil as affected by light, antioxidants and temperature. **J. Food Process Preserv.** 28: 1-10.

SAS Institute. 1996. **SAS User's Guide**. The SAS Institute, Cary, NC.

Sidel, J.L. and H. Stone. 1983. An introduction to optimization research**. Food Tech.** 37(11): 36-38.

Sevatson, E. and G.R. Huber. 2000. Extruder in food industry, pp. 167-204. *In* M.N. Riaz, ed. **Extruder in Food Applications.** Technomic Publising Co., Inc., Pennsylvania. 223p.

Shahidi, F. and U.N. Wanasubdara. 1998. Omega-3 fatty acids concentrate: nutritional aspects and production technologies. **Trends Food Sci Tech.** 9: 230-240.

Shin, T.S., J.S. Godber, D.E. Martin and J.H. Wells. 1997. Hydrolytic stability and changes in E vitamers and oryzanol of extruded rice bran. **Food Chem.** 23: 177-191.

Simopoulos, A.P. 2000. Symposium: Role of Poultry Products in Enriching the Human Diet with n-3 PUFA, human requirement for n-3 polyunsaturated fatty acids. **Poultry Sci.** 79(7): 961-970.

_____. 2002. Omega-3 fatty acids in inflammatory and autoimmune diseases. **J. Am. Coll. Nutr.** 21(6): 495-505.

Singh, N., P. Cairns, V.J. Morris and A.C. Smith. 1998. Physical properties of extruded wheat starch-additive mixtures. **Cereal Chem.** 75(3): 325-330.

Skerrett, P.J. and C.H. Hennekens. 2003. Consumption of fish and fish oils and decreased risk of stroke. **Prev. Cardiol.** 61(1): 38-41.

SPSS Inc. 2004. Statistical Package for the Social Science (SPSS®) version 12. SPSS Inc., Chicago

Stone, N.J. 1997. Fish consumption, fish oil, lipids, and coronary heart disease. **Am. J. Clin. Nutr.** 65: 1083-1086.

Stone, W.L. and A.M. Papas. 1997. Tocopherols and the etiology of colon cancer. **J. Natl. Cancer Inst.** 89(14): 1006-1014.

Strange, E.D. and K. Shaich. 2000. Extraction of lipids from extruded corn-soy blends. **Lipids** 7(4): 217-224.

Suknark, K., J. Lee, R.R. Eitenmiller and R.D. Phillips. 2001. Stability of tocopherols and retinyl palmitate in snack extrudates. **J. Food Sci.** 66(6): 897-902.

_____, K.H. McWatters and R.D. Phillips. 1998. Acceptability by American and Asian consumers of extruded fish and peanut snack products. **J. Food Sci.** 63(4): 721-725.

_____, R.D. Phillips and Y.-W. Huang. 1999. Tapioca-fish and tapioca-peanut snacks by twin-screw extrusion and deep-fat frying. **J. Food Sci.** 64(2): 303-308.

Tappel, A.L. 1992. Vitamin E, pp.179-203. *In* G.F. Combs, Jr., ed. **The Vitamins: Fundamental aspects in nutrition and health.** Academic Press, Inc., San Diego.

Terry, P., P. Lichtenstein, M. Feychting, A. Ahlbom and A. Wolk. 2001. Fatty fish consumption and risk of prostate cancer. **LANCET** 357: 1764-1766.

The Natural Marketing Institute (NMI). 2002. Available source: http://www.nutraceuticalsworld.com/nov.022.htm**,** July 22, 2003.

Tuley, L. 1992. The rice evaluation. **Food Rev.** 19(5): 13-14.

Unlu, E. and J.F. Faller. 2002. RTD in twin-screw food extrusion. **J. Food Eng.** 53: 115-131.

USDA. 2004. **National Nutrient Database for Standard Reference, Release 16-1.** Nutrient Data Labboratory. Avialable Source: http://www.nal.usda.gov/fric/ foodcomp/search, October 12, 2004.

Venugopal, V. and F. Shahidi. 1998. Traditional methods to process underutilized fish species for human consumption. **Food Rev. Int.** 14(1): 35-97.

Wagner, K-H., F. Wotruba and I. Elmadfa. 2001. Antioxidative potential of tocotrienols and tocopherols in coconut fat at different oxidation temperature. **Eur. J. Lipid Sci. Tech.** 103: 746-751.

Weisman, D., M. Motro, E. Schwammenthal, E.Z. Fisman, A.Tenenbaum, D. Tanne and Y. Adler. 2004. Efficacy of omega-3 fatty acid supplementation in primary and secondary prevention of coronary heart disease. **IMAJ.** 6: 227-232.

Wennermark, B., H. Ahlmen and M. Jagerstad. 1994. Vitamin E retention by using freshly milled whole-meal wheat-flour during drum-drying. **J. Agric. Food Chem.** 46(2) 1348-1351.

Whelton, S.P., J. He, P.K. Whelton and P. Muntner. 2004. Meta-analysis of observational studies on fish intake and coronary heart disease. **Am. J. Cardiol.** 39: 1119-1123.

Ye, L., W.O. Landen and R.R. Eitenmiller. 2000. Liquid chromatography analysis of all-trans-retinyl palmitate, β-carotene and vitamin e fortified foods and the extraction of encapsulated and nonencapsulated retinyl palmitate. **J. Agric. Food Chem.** 48: 4003-4008.

Yeh, A.-I., S.-J. Hwang and J.-J. Guo. 1992. Effects of screw speed and feed rate on mean residence time distribution and axial mixing of wheat flour in a twin-screw extruder. **J. Food Eng.** 17: 1-13.

Yeh, A-I. and Y-M. Jaw. 1999. Effects of feed rate and screw speed on operating characteristics and extrudate properties during single-screw extrusion cooking of rice flour. **Cereal Chem.** 76(2): 236-242.

APPENDICES

Appendix A

Consumer survey questionnaire

Consumer survey questionnaire conducted in Thailand
(Translated from Thai to English)

Consumer survey questionnaire

Project

"Development of Snack Supplemented with Protein and Omega-3 Fatty Acids"

This survey is a part of the research of Miss Nantipa Pansawat, graduate student in the Department of Agro-Industry Product Development, Faculty of Agro-Industry, Kasetsart University, Bangkok. Please feel free to answer the questions. Your name and personal identification is not required.

Explanation

"Snack" in this questionnaire refers to snacks made from starchy ingredients that may have different flavors and be consumed between meals.

Thanks for your time and the participation

Please make ✓ in front of the answer that represent yourself or your opinion

1. Gender

❍ Male ❍ Female

2. Age

❍ Less than 15 yr ❍ 15-20 yr

❍ 21-25 yr ❍ 26-35 yr

❍ 35-45 yr ❍ Over 45 yr

3. The highest education

❍ Primary school ❍ Middle school

❍ High school or equivalent ❍ Certificate from 2 years college

❍ 4 Years college ❍ Graduate school or higher

4. Occupation

❍ Housewife ❍ Government employee

❍ Company employee ❍ Own business/ self employed

❍ Labor ❍ Agriculture

❍ Student ❍ Others, please specify…….

5. Your income per month

❍ Less than 5,000 Baht ❍ 5,001-7,000 Bath

❍ 7,001-10,000 Baht ❍ 10,001-15,000 Baht

❍ 15,000-20,000 Baht ❍ 20,001-25,000 Baht

❍ 25,001-30,000 Baht ❍ 30,000 Baht and more

6. What kind of food you usually have between meals?

(You can answer more than one.)

❍ Thai desert

❍μ Bakery products

❍ Snack made from starchy material

❍ Thai style between meals food

❍ Potato chips

❍ Fruits

❍ Nuts, seeds eg. dry pumpkin seed, dry watermelon seed

7. How often do you eat snack?

- ❍ Everyday
- ❍ 2-3 times a week
- ❍ Once a week
- ❍ 2 times a month
- ❍ Once a month
- ❍ Less than once a month

8. Main reasons for eating snack (you can answer more than one)

- ❍ Between meals
- ❍ Instead of meal
- ❍ Eat while drinking
- ❍ Eat when has free time
- ❍ Eat while traveling
- ❍ Eat while watching TV
- ❍ Eat occasionally with different reason

9. What time do you usually have snack?

- ❍ Before 10:00AM
- ❍ 10:00AM-1:00PM
- ❍ 1:00PM-5:00PM
- ❍ 5:00PM-8:00PM
- ❍ After 8:00PM

10. How much do you usually pay for a snack?

- ❍ 5 Baht
- ❍ 6-10 Baht
- ❍ 11-15 Baht
- ❍ 16-20 Baht
- ❍ 21-25 Baht
- ❍ more than 25 Baht

11. Where do you usually buy snack?

- ❍ Convenience store eg. Seven-Eleven, AM-PM, Starmart
- ❍ Local store
- ❍ Supermarket eg. Tops, supermarket in shopping mall, Lotus
- ❍ Wholesale store
- ❍ Other, please specify……..

12. What Taste/ flavor of the snack do you prefer?

- ❍ Original flavor of the raw material
- ❍ Salty
- ❍ Sweet
- ❍ Strong flavor and spicy

13. What kind of texture of the snack that you like?
    - ❍ Puff, very low density
    - ❍ Puff, more dense
    - ❍ Lightly puff, dense
    - ❍ Dense, brittle
    - ❍ Dense and hard
14. What do you think about the nutritional value of the snacks available in the market?
    - ❍ No nutritional value
    - ❍ Low nutritional value
    - ❍ Moderate nutritional value
    - ❍ High nutritional value
    - ❍ I have no idea
15. How do you like food that contains fish?
    - ❍ Like extremely
    - ❍ Like very much
    - ❍ Like moderately
    - ❍ Like slightly
    - ❍ Neither like of dislike
    - ❍ Dislike slightly
    - ❍ Dislike moderately
    - ❍ Dislike very much
    - ❍ Dislike extremely
16. Have you ever heard about the heath beneficial effects of fish oil?
    - ❍ Yes
    - ❍ No
17. Do you know the information about EPA and DHA?
    - ❍ Yes
    - ❍ No
18. What is the source of your information according to question 12 and 13?
    - ❍ TV or radio
    - ❍ Magazine
    - ❍ Scientific publication
    - ❍ Leaflet, brochure
    - ❍ Food exhibition
    - ❍ Friend or personal contact
19. Have you ever had food, snack or supplement that labeled EPA and DHA?
    - ❍ Yes
    - ❍ No
20. Have you ever had snack containing fish oil?
    - ❍ Yes
    - ❍ No

21. Are you willing to buy snack that contain fish oil?

❍ Yes ❍ No

Thank you

Appendix B

Instruction for the sensory test

Instruction for the sensory test

Prior the test, all participants will be asked to read and sign the consent forms. Participant will be assigned random digits code number for subsequent identification. Each participant will be provided with plastic utensils, napkins, water, unsalted cracker and samples. The panelist will be presented with five samples and asked to rate attributes of the samples on ballots (evaluation forms)

The sample will be served to the panelist one at a time. The panelists will be asked to test the samples and rated for the appearance, color, aroma, flavor, texture ad overall acceptability. The attribute is rated on a nine-point hedonic scale (See the evaluation form).

The panelists will be asked to mark in the space that best describes how they feel about the sample and eat a piece of cracker and rinse your mouth with water between samples.

Appendix C

Consent form

Consent form for sensory test in at the University of Georgia, Athens, GA

I, _____, agree to participate in the research entitled "Development of an Extruded Snack Supplemented with Fish Protein and omega-3 Fatty Acids" which is being conducted by Ronald R. Eitenmiller and Nantipa Pansawat of Department of Food Science and Technology, Athens, the University of Georgia, phone number (706) 542-1091, (706) 543-7505

I understand that participation is voluntary and whether or not I participate will not affect how I am treated. I can withdraw my consent at any time and have the results of the participation returned to me, removed from the experiment records, or destroyed.

The following points have been explained to me:

1) The reason for the research is to gather information on consumer acceptability of the extruded snacks supplemented with protein and omega-3 fatty acid.

2) The procedures are as follows: Coded samples will be placed in front of me and I will evaluate them by normal standard method (tasting, swallowing, rinsing, expectorating) and indicate my evaluation on the score sheets. All procedures are standard methods as published by the American Society for Testing Materials. It is estimated that each participant will use less than 15 minutes to evaluate the five products

3) Participation entails the following risks: The risk which can be envisioned is that of an allergic reaction to rice flour, fish or fish oil. However, because the nature of the products will be known to me beforehand, the situation can normally avoided. In the event that my participation in this study results in a medical problem, the researcher will call the emergency services and treatment will be made available. My insurance company or I will be billed for the costs of any such treatment. No provision has been made for payment of these costs or to provide me with any other financial compensation.

4) It is my responsibility to make known any food allergies generally and any allergies I may have against the products being test, like fish, fish oil, rice flour and vitamin E to the investigators

(Allergies: _____)

5) The results of this participation will be confidential and will not be released in any individually identifiable form without my prior consent unless required by law.

6) The investigators will answer any further questions about the research, either now or during the course of the project.

I understand that I am agreeing by my signature on this form to take part in this research and understand that I will receive a signed copy of this consent form for my records.

_____ _____

Signature of Investigation Signature of Participant

Date: _____ Witness: _____

Additional questions or problems regarding your rights as a research participant should be addressed to Chris A. Joseph, Ph.D. Human Subjects Office, University of Georgia. 606A Boyd Graduate Studies Research Center, Athens, Georgia 302-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu

Appendix D

Evaluation form for the acceptance test

Evaluation form for the acceptance test

Please evaluate this sample and mark in the space that best describes how you feel about this sample.

The samples will be served to you one at a time. Please eat a piece of cracker and rinse your mouth with water between samples.

Sample Code_____

1. How do you like the "APPEARANCE" of this sample?

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

2. How do you like the "COLOR" of this sample?

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

3. How do you like the "AROMA" of this sample?

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

4. How do you like the "FLAVOR" of this sample?

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

5. How do you like the "TEXTURE" of this sample? (crispiness, crunchiness, hardness)

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

6. How do you like the "OVERALL ACCEPTABILITY" of this sample?

Dislike Extremely [ ] 1	Dislike Very much [ ] 2	Dislike Moderately [ ] 3	Dislike Slightly [ ] 4	Neither Like nor Dislike [ ] 5	Like Slightly [ ] 6	Like Moderately [ ] 7	Like Very much [ ] 8	Like Extremely [ ] 9

7. Will you buy this product if it is commercially available?

[ ] Yes [ ] No

8. Please briefly describe any outstanding characteristics of this sample_____

_____

_____

Thank you

CURRICULUM VITAE

NAME: Miss Nantipa Pansawat

BIRTH DATE: October 18, 1975

BIRTH PLACE: Pranakorn Sri Ayutthaya, Thailand

EDUCATION:

YEAR	INSTITUTION	DEGREE
1996	Kasetsart Univ.	B.Sc. (Fisheries)
2000	Kasetsart Univ.	M.Sc. (Fishery Products)

POSITION: Lecturer

WORK PLACE: Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

SCHOLARSHIP: The Ministry of University Affairs, Thailand