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INVESTIGATION OF THE PROPERTIES OF MACARONI ENRICHED WITH WHEAT GERM

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by

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ABSTRACT

INVESTIGATION OF THE PROPERTIES OF MACARONI ENRICHED WITH WHEAT GERM

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Ph. D in Food Engineering
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In this study, shelf life of whole wheat germ and wheat germ oil was studied and enriched macaroni was produced using raw and microwaved wheat germ. Accelerated shelf life test at 60, 70 and 80 C was used for determination of shelf life of whole wheat germ. Also shelf life of whole wheat germ was studied at 30 and 40 °C and these results were compared with results of accelerated shelf life test. Shelf life studies of wheat germ oil at 30 and 40 °C revealed the fact that whole wheat germ was more stable to oxidative and hydrolytic changes than wheat germ oil.

Macaroni was produced from semolina and semolina blended with 15% raw or microwaved wheat germ which their shelf life was determined and stored at room temperature for one year. Samples were analyzed for sensory attributes, cooking quality, color, compressibility, microbial count, *in-vitro* protein digestibility and gelatinization of starch during storage. Effect of cooking time (10, 20, 30 min) on cooking quality of macaroni was also studied.

Results indicated that protein content of macaroni increased up to 17% with wheat germ addition, as expected. Addition of raw and microwaved wheat germ increased cooking losses and volume of cooked macaroni but reduced weight after cooking. No significant effect ($P<0.05$) of storage was found on the measured parameters except acidity. Acidity of enriched macaroni samples showed a significant increase during storage. Supplementing durum semolina with raw and microwaved wheat germ provided macaroni with similar results to durum macaroni in compressibility and *in-vitro* protein digestibility. Enriched macaroni samples were more red and less yellow in color compared to the control sample.

Differential scanning calorimetry (DSC) was used to study the extent of gelatinization of starch in macaroni samples and two endothermic transitions were observed. Control macaroni significantly shifted to higher onset (To_1) and peak temperature (Tp_1), narrow gelatinization range (T_1) and lower gelatinization enthalpy (H_1) when compared with semolina. Addition of raw and microwaved wheat germ in to macaroni increased To_1, but lowered the T_1 and H_1 of the macaroni significantly. The second endothermic DSC peak indicated the increase in amylose-lipid complexation (H_2) of macaroni after enrichment with raw and microwaved wheat germ.

In taste panel evaluations, significant difference was found between control and enriched types of macaroni in flavor and appearance (degree of difference was found to be slight) but not in texture and overall scores.

Microbial counts of macaroni samples were followed by measuring total number of bacteria, mold and yeast at initial, after six and twelve mounts of storage period. It was found that macaroni samples were safe to eat after one-year storage.

Keywords: wheat germ, macaroni, shelf life, cooking quality, sensory evaluation, compressibility, protein digestibility, starch gelatinization, differential scanning calorimetry (DSC).

ÖZ

BUĞDAY RUŞEYMİ İLE ZENGİNLEŞTİRİLMİŞ MAKARNANIN ÖZELLİKLERİNİN İNCELENMESİ

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Bu çalışmada buğday ruşeymi ve buğday ruşeymi yağının raf ömrü çalışıldı ve daha sonra çiğ ve mikrodalgada kavrulmuş ruşeym eklenerek zenginleştirilmiş makarna üretildi. Buğday ruşeyminin raf ömrü hızlandırılmış raf ömrü metoduyla 60, 70 ve 80 C de belirlendi. Aynı zamanda buğday ruşeyminin 30 ve 40 C deki raf ömrü çalışıldı ve sonuçlar hızlandırılmış raf ömrü metoduyla bulunan sonuçlarla kıyaslandı. Buğday ruşeymi yağının 30 ve 40 °C de yapılan raf ömrü çalışması sonuçları buğday ruşeyminin yağına göre hidrolitik ve oksidatif bozulmaya daha dayanıklı olduğu gerçeğini ortaya koydu.

İrmikten ve irmiğe %15 oranında raf ömrü belirlenen çiğ ve mikrodalgada kavrulmuş ruşeym eklenerek makarna üretildi ve bir yıl oda sıcaklığında depolandı. Elde edilen makarna örneklerinin depolama süresindeki duyusal nitelikleri, pişme kalitesi, rengi, sıkıştırılabilirliği, mikroorganizma sayısı, vitro da protein sindirilebilirliği ve nişasta jelatinizasyonu belirlendi. Aynı zamanda pişme süresinin (10, 20 ve 30 dak) makarnanın pişme kalitesine etkisi de incelendi.

Sonuçlar beklendiği gibi makarna proteinin ruşeym eklenmesiyle %17 ye kadar yükseldiğini gösterdi. Çiğ ve mikrodalgada kavrulmuş ruşeym eklendiğinde, pişmiş makarnanın hacminin ve suya geçen madde miktarının arttığı, fakat ağırlığının azaldığı bulundu. Asitlik dışında diğer ölçülen parametrelere depolama süresinin anlamlı bir etkisi olmadığı bulundu. Zenginleştirilmiş makarnaların asitliği depolama sırasında anlamlı bir artış gösterdi. Durum makarnasıyla ham ve mikrodalgada kavrulmuş ruşeym eklenerek elde edilmiş makarnaların sıkıştırılabilme ve protein sindirilebilirliği arasında anlamlı bir fark bulunmadı. Zenginleştirilmiş makarnaların kontrol makarnası ile kıyaslandığında daha kırmızı ve daha az sarı olduğu belirlendi.

Makarna örneklerindeki nişastanın jelatinizasyon miktarını incelemek için diferansiyel tarayıcı kalorimetresi (DSC) kullanıldı ve iki endotermik geçiş gözlendi. Kontrol makarnası irmikle kıyaslandığında daha yüksek başlangıç (To_1) ve pik sıcaklığına (Tp_1), daha dar jelatinizasyon aralığına (T_1) ve daha düşük jelatinizasyon entalpisine (H_1) sahip olduğu bulundu. Ham ve mikrodalga ile kavrulmuş buğday ruşeymi eklenmesi makarnanın To_1'ini arttırmış, fakat T_1 ve H_1 'i anlamlı bir şekilde düşürmüştür. İkinci endotermik DSC piki, ham ve mikrodalga ile kavrulmuş buğday ruşeymi eklenmesi ile makarnanın amiloz-yağ kompleksinin (H_2) artığını göstermiştir.

Test panel değerlendirmesinde, kontrol ve zenginleştirilmiş makarna çeşitlerinin tat ve görüntülerinde anlamlı bir fark ($P<0.05$) bulundu (derece farkı az olarak bulundu) fakat yumuşaklık/sertlik derecesi ve genel skorda bir fark bulunmadı.

Makarna örneklerindeki mikroorganizma yükü toplam bakteri, küf ve maya sayıları başlangıçta, altı ay ve on iki ay sonra ölçülerek takip edilmiştir. Sonuçlar bir yıl sonunda makarna örneklerinin güvenle tüketilebileceğini göstermiştir.

Anahtar Kelimeler: buğday ruşeymi, makarna, raf ömrü, pişme kalitesi, duyusal değerlendirme, sıkıştırılabilme, protein sindirilebilirliği, nişasta jelatinizasyonu, diferansiyel süzme kalorimetresi (DSC).

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To my family

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PV	Peroxide value (meq/kg)
FFA	Free fatty acid (%)
TBA	Thiobarbutiric acid (mg malonaldehyde/kg)
C	Concentration (meq/kg)
T	Time (day)
k	Rate constant ($day^{-1)}$
k_0	Pre-exponential rate constant
E_a	Activation energy
R	Gas costant (8.314 J/mol.K)
T	Absolute temperature (K)
S	Durum semolina
RG	Raw wheat germ
MG	Microwaved wheat germ
CM	Control macaroni produced with 100% durum semolina
RM	Macaroni produced with 15% raw wheat germ and 85% durum semolina
MM	Macaroni produced with 15% microwaved wheat germ and 85% durum semolina
L	Hunter brightness index
a	Hunter redness index
b	Hunter yellowness index
IVPD	*In-vitro* protein digestibility
T_o	Onset temperature (°C)
T_c	Conclusion temperature (°C)
T_p	Peak temperature (°C)
ΔH	Enthalpy (J/g)
ΔT	Melting range (T_c-T_o) (°C)
APC	Aerobic plate count

CHAPTER I
INTRODUCTION

1.1. Pasta Raw Materials

Pasta products have been known to Mediterranean civilizations for many centuries, and are eaten and enjoyed everywhere. Pasta is popular because it is a versatile, natural and wholesome food, and the manufacturing process is relatively simple. Pasta products are available in a variety of shapes, can be stored conveniently for long periods, are easily transported, and have excellent nutritional and hygienic quality (Agnesi, 1996).

The raw materials used to manufacture pasta must perform the following functions;

- allow the pasta to be shaped by extrusion through a die;
- yield a strong, flexible, dry finished product free from checking with good packaging tolerance and good storage stability;
- yield a translucent dry finished product with the essential aesthetic qualities of a smooth clear surface free of specks and a bright amber yellow color;
- yield a firm, resilient, nonsticky cooked product with a pleasing taste and aroma;
- make a significant contribution to the balanced diet of the consumer;
- be beyond reproach with respect to hygiene (Feillet and Dexter, 1996).

Pasta is the most commonly consumed paste-product made from durum wheat. The term pasta has generally been reserved to describe paste products fitting the "Italian" style of extruded foods such as spaghetti or lasagne, and is usually distinguished from wheat other than durum. Pasta in Italian tradition can be categorized into four main groups or types: long-goods, short goods, egg noddles, and specialty items. Long-goods include such products as spaghetti, vermicelli, and linguine; short-goods include elbow macaroni, rigatoni, and ziti; egg noodles consist of pasta made with eggs; and specialty items include lasagne, manicotti, jumbo shells, and stuffed pasta.

Many additional products fit into one or more of these categories. The name of a specific pasta product usually denotes the product's shape, size, and thickness (Dick and Matsuo, 1988).

1.2. The Components of Semolina That Effect Pasta Quality

1.2.1. Proteins

The protein component of wheat changes according to the varieties, the areas of cultivation, hydroclimatic conditions of the farming years, techniques of cultivation, methods and duration of ensilage, etc. The average percentage is approximately 13%, with concentrations that grow in the surface layers of the kernel and in the layers immediately adjacent to the germ. The proteins of wheat mostly comprise gliadin and glutenin (approximately 80%), the rest is composed of albumin, glaidin and free amino acids. Gliadin and glutenin are commonly considered the "star" proteins of pasta, at least as regards its features that consumers most highly appreciate (cooking capacity, elasticity, "al dente" chewability). Actually their role in these characteristics is decisive, but it is also necessary to add that they do not have an exclusive merit in this and that, additionally, they also perform other technologically fundamental functions, among which water absorption is very important. When water is added to the semolina (or to the flour) and the dough is mixed mechanically, glutenin and gliadin form gluten, a protein compound that, as every pasta maker in the world knows (or should know...), forms a kind of mesh in the structure of the dough, trapping the starch grains and basically preventing the pasta during cooking from turning into polenta. Actually this gross simplification does injustice to the professionalism of the pasta maker, who needs to know many other things. For example, the mechanism of gluten formation and its way of behaving with water is fundamental knowledge also to be able to dry pasta well (Martinella, 2004).

Gluten is extremely hungry for water: it absorbs twice its own weight and moreover tends to hold it through complex chemical bonds. Its hunger for water moreover cannot be completely satisfied in the forming the dough, since for technological reasons the moisture of the dough normally cannot exceed the limit of 35%, or it suffers huge problems in extrusion. In any case, gluten, in relation to water, behaves as an antagonist for starch, since it tends to take up not only a greater amount of it, but also more quickly once its formation has begun. And here we need to make a consideration: the percentage of gluten (gliadin and glutenin) and starches in the semolina (or the flour) in some way rebalance the quantities of water absorbed by these two antagonists: gluten absorbs 200% of its own weight, starch (not damaged...) approximately 35-50%. Since however starch is present in semolina in a quantity of approximately five times greater than that of gluten, the water of the dough is equally divided between them. Since this "competition" between gluten and starch is particularly obstinate, if the water is not uniformly distributed already at the initial moment of the formation of the dough it is very difficult for it to then be able to be transferred from one to the other antagonist, in particular from the gluten to the starch (Martinella, 2004).

It was noticed that the capacity of gluten to hold water depends on its quality: this is the main reason why durum wheat semolina pasta prevails over that of soft wheat flour pasta in tenacity, cooking capacity and less stickiness. The gluten of soft wheat flour, in fact, even if it is able to absorb more water than that of semolina, it is less able to hold it. Superior capacity of the gluten to hold water compared to starch obviously has to affect the drying of the pasta: pasta makers well know that when they use semolina very rich in proteins (and so with high quality gluten) they have to work harder to dry the pasta properly. But aside from this observation, the practical consequences are also others: for example the tendency of the gluten to "follow" the moist zones of the product during pre-drying, since its "mobility" is obviously possible when the pasta is still in the plastic state, so with a high water content, let's say no lower than 25%. The water absorption of gluten is not linear: it is very slow at the start of processing the dough, but it grows quickly as the development of the gluten is perfected. The last few minutes of dough formation are when the swelling of the gluten is most accentuated (and also its elasticity). Since in the technology of pasta, the development of gluten is not, as we have already pointed out, complete, shortening the dough time can have negative consequences, since the rheologic characteristics of the semolina will not be exploited to the best; but also lengthening them too much does not do the pasta any good. If the mechanical action of the kneading machine continues when the gluten has already absorbed all the available water and so its development has stopped, the forces applied on the dough and the consequent stresses will damage it, reducing its elasticity and strength (Martinella, 2004).

Wheat proteins are a highly heterogenous material, including albumins (soluble in water), globulins (soluble in neutral salt solutions), gliadins (soluble in 70% ethanol and in acids), and glutenins (soluble in acids, bases, hydrogen, and hydrophobic bond-breaking solvents). Albumins and globulins are cytoplasmic proteins with enzymic activities and foaming emulsifying properties. Gliadins with their high extensibility, and glutenins with their high elasticity are associated with lipids and other components (minerals, carbohydrates) in a unique, viscoelastic protein complex referred to as gluten. The relaxed gluten of durum wheat appears to be more porous than the gluten of bread wheat. It was showed that the proportion of soluble proteins decreased significantly with increasing protein content; increase in total protein was completely accounted by an increase in the amount of gluten proteins (Feillet, 1998).

From the analysis by electron microscopy, it was concluded that most of the protein in the durum wheats can be found with in the cell structure, surround the starch granules as enveloping proteins, with lesser amounts found as fibril proteins or deposited as protein mass; proteins are deposited in the matrix differently in different varieties (Feillet, 1998).

Besides, it is well recognized that the vitreousness of kernel is positively correlated with the protein content, which appears to have a slight negative influence on semolina yield (Feillet, 1998).

The firmness of cooked pasta and the surface condition of pasta after cooking (which is known to be related to loss of matter in cooking water and tendency to stickiness) are the two main characteristics on which cooking quality depends (Feillet, 1998).

There is a very consistent relationship between the protein contents of wheat and semolina and the cooking quality of pasta: a wheat protein content higher that 13% was reported to yield a satisfactory final product, whereas protein content lower than 11% gave a very poor product. Gluten properties were also identified as an essential factor of cooking quality. Cooked pasta resilience is largely governed by gluten characteristics; using viscoelastograph, it was demonstrated that strong gluten varieties with high elastic recovery exhibit good cooking quality, whereas weak gluten varieties with low elastic recovery have poorer cooking quality. Firmness of cooked pasta is related to gluten strength of durum wheat (Feillet, 1998).

As is the case for bread technology and common wheat proteins; it is still not clearly known which gluten components are favorable and which are unfavorable to pasta cooking quality. At the present time, results of many workers support the following major conclusions:

1. Durum wheat varieties that possess the 45-γ gliadin fraction have a high intrinsic cooking quality, whereas those that posses the 42-γ gliadin have a poorer cooking quality.
2. Superior cooking quality is linked to a high glutenin-glaidin ratio or a high percentage of "insoluble" proteins.
3. The role of glutenin subunits is controversial.

Studies showed that the presence of the 45-γ component was linked to a strong gluten and therefore to a high-quality wheat variety; the presence of the 42-γ component was linked to a weak gluten and a poor-quality variety. A comparison of the protein fractionation led to conclude that durum wheat varieties with a high content of glutenins (and albumins) tend to show good cooking quality. It was observed that high wheat ash content was associated with strong gluten characteristics (Feillet, 1998).

The protein factors responsible for the firmness and the stickiness of cooked pasta are different. High protein and gluten viscoelasticity (and therefore the presence of proteins linked to 45- γ gliadin) would be responsible for the firmness of cooked pasta. In a preliminary report, the resistance of pasta to disintegration during cooking in boiling water and the amount of protein left in the spaghetti cooked in basic medium was linked. A high ratio of isopropanol-soluble proteins (in presence of reducing agent) to water-soluble proteins would prevent stickness, as would a high sulfhydryl-plus-disulfide content of glutenins (Feillet, 1998).

Proportion of insoluble proteins in cooked pasta appears to be related to cooked pasta quality, support the hypothesis that the ability of pasta proteins to form, during cooking, an insoluble network able to entrap the swollen starch granules could be partially responsible for the quality of cooked pasta (Feillet, 1998).

High hydrophobicity of gluten proteins would prevent the penetration of water into pasta during cooking and therefore prevent swelling, surface of disintegration, and stickiness. A compact protein structure with inaccessible hydrogen bonds (less dispersible in urea) could result in formation of a more stable protein network during processing (Feillet, 1998).

Much evidence (arising from physicochemical, genetic, and genetic, and technological studies) supports the conclusion that both protein quantity and quality are involved in determining cooked pasta characteristics, i.e., firmness, surface condition, and stickiness after cooking and overcooking (Feillet, 1998).

The ability of proteins to form an insoluble network able to entrap the swollen and gelatinized starch granules and to avoid disruption of the spaghetti surface and leaching of carbohydrates and proteins by boiling water is probably the basis of good cooking quality in durum wheat (Feillet, 1998).

This ability mainly dependent on two factors: protein quantity and protein quality. It is easy to understand why high protein content is favorable to good quality; the polypeptide chains are more numerous and chances for proteins to interact and to form a resistant network are higher (Feillet, 1998).

Protein quality is still more a theoretical concept than a physicochemical and experimental-based reality. Although the roles of 45- γ gliadin and insoluble glutenin materials are well documented, much more research is needed to identify the nature of components and the mechanisms of the phenomena involved in pasta cooking quality (Feillet, 1988).

1.2.2. Starch

In flour and semolina, starch is quantitatively the main component (on average around 60-65%). Chemically it is a glucidic substance, composed of microscopic grains of diameter between 2 and 100 thousandths of a millimetre, comprising approximately 25% amylose (a linear chain of glucose units) and 75% amylopectin (chain with a branched structure of glucose units). These two components of starch are physically combined in a crystalline kind of reticular structure. In plain words, starch is a compound of sugars, grouped together in its two main fractions, amylose and amylopectin. Starch is insoluble in water, while its two essential fractions have differentiated behaviours: amylose is soluble in hot water, amylopectin is not. Anyhow, in the presence of water and due to the aggression of the specific hydrolytic enzymes (amylase), some of the chemical bonds that in starch keep the sugars of the chains united break, freeing or forming products of demolition, in particular dextrins, maltose and glucose. We should note that these are the compounds, especially the dextrins, that are mostly responsible for the surface stickiness of pasta. They are also the cause of its typical sweetish flavour. The attack on the starch by the amylase enzymes is aided by the temperature, but also by the damage of its grains that is inevitably caused by grinding the wheat. Damaged starch therefore ends by "freeing" part of those sugars that comprise its molecular structure, a phenomenon that is exploited - as everyone knows - in the fermentation of doughs for the production of bread. But when semolinas (or flours) are used to prepare doughs for the production of pasta, the matter changes: the excessive presence of sugars in the pasta is always solely damaging. During the drying some sugars (pentosans) can set off the complex reaction of Maillard, which develops in several phases and has the consequence of non-enzymatic blooming of the product, the loss of the nutritional value of the pasta due to some of its amino acids getting blocked and, in the most striking cases, even the onset of anomalous tastes and smells. Apart from the Maillard reaction, the sugars in the dough (and in the pasta to be dried) cause, as we will see, other effects that are not always secondary and negligible. The level of damage to the starch grains of the semolina (or of the flour) has direct consequences also as regards the behavior of the starch itself with the water: integral starch absorbs, for example, less than half its weight, whereas damaged starch, instead, up to double. The absorption of water by the starch also varies with the changing temperature of the dough, a phenomenon that is well known to skilled pasta makers and is on the contrary exploited to improve the overall quality of the dried product, depending on its shape or particular characteristics of the cut of the pasta at the die outlet (Martinella, 2004).

Durum starches were in general more susceptible to diastase and gave higher viscosity values than hard red spring wheat starches. Also swelling capacity of durum starches was greater than that of starches. Starches from durum wheat generally had higher water-binding capacities, greater rates of iodine absorption, and slightly lower temperatures of initial pasting than starches from other wheat classes. These results indicate that durum whet starch begins to swell at a slightly lower temperature than starch from other wheat classes. This observation may be related to the suggested less-compact granule structure of durum starch. Consequently, solvents penetrate durum starches more easily than other wheat starches (Fortini, 1988).

Gelatinization data for durum starches isolated from semolina and spaghetti showed that semolina starch had a higher peak viscosity than the starch isolated from spaghetti. This decrease in peak viscosity as a result of processing indicated that starch damage occurred to some extent during processing (Fortini, 1988).

The results of experiments on water binding capacity and starch damage revealed that the percentage of damaged starch was greatly increased during pasta processing. Certain variability among the different wheat varieties was noted, but in all cases the amount of starch damage in spaghetti was much higher than in the corresponding semolina. Further experiments showed that some starch was damaged during the extrusion phase of processing, but the greatest change occurred during drying step.
Although starch may represent up to 80% of semolina dry matter, it has not been studied as deeply as protein, at least with regard to the evaluation its role in determining the quality of alimentary pasta. This lack of interest may be attributed to the opinion that a substance as chemically uniform as starch cannot have the high reactivity characteristics of proteins. Attempts to correlate the starch content of semolina with the cooking firmness of the pasta obtained have always indicated a negative correlation. However, the validity of such correlation is uncertain because of the high negative correlation between protein and starch (Fortini, 1988).

Differences in quality among semolina samples derived from different varieties of durum wheat are due to greater or lower capacities of their proteins to form, during pasta-making, a network capable of retaining the other components of semolina, especially starch granules. A protein network that is to loose allows starch granules to escape during cooking. A starch layer forms on the surface of pasta, making it sticky and giving strands a tendency to clump together (Fortini, 1988).

1.2.3. Enzymes

Enzymes of durum wheat that are known to be the most technologically important with regard to the characteristics of color and cooking quality in pasta products are α-amyloses, lipoxygenases, peroxidases, polyphenoloxidases, and proteases.

Whereas, the importance of enzymes is quite different, both for the formation of the dough and the drying of the product. The most significant ones are, as we already mentioned, the amylases, enzymes that break the chains of starch, freeing dextrins (stickiness of the pasta) and maltose (reducing sugars that increase the possibility of the Maillard reaction). However other enzymes affect the drying of the pasta: for example protease, that hydrolizes proteins and, therefore, weakens the gluten. Lipoxidase and lipoxigenase, responsible for the oxidation and the destruction of the vitamins that help produce the yellow colour of pasta (tocopherols and, above all, carotenes).

All these enzymes have different sensitivity to heat. The beta-amylases (which free maltose from the amylopectin of starch) are already deactivated at 50°C, while the alpha-amylases (which free dextrins from the amylose of starch) and the lipoxidases are active well beyond 65°C, becoming active precisely when the temperature of the pasta exceeds 50°C. Since the only way of avoiding the action of the enzymes is their deactivation by coagulation, the temperature both of the dough, but above all that of the pasta during drying, particularly when it is still very moist (pre-drying), becomes decisive. The alpha-amylases, lipoxidases and lipoxigenases can be deactivated (at least partially) only with effective hydrothermal treatment of the pasta in the pre-drying phase. And this is the only way of protecting the yellow pigments of semolina (carotenoids) and avoiding the grey colouring of the dried end product, due to the formation of brown melanin in the pasta. Obviously also the formation of the dough in a vacuum helps, since all the reactions triggered by these enzymes require oxygen. But the best results (no surface stickiness and a bright yellow colour of the pasta) can only be obtained with an energetic hydrothermal treatment of the pasta during the pre-drying phase (Martinella, 2004).

a) α-Amylases

α-Amylases, whose activity in cereals has been recently surveyed, are endosplitting enzymes that hydrolyze the α-1,4-glycosidic bonds of starch molecules are derived polysaccharides in a more or less random fashion. They are absent from mature grain and appear upon germination. For this reason, works on durum wheat α-amyloses are associated with the study of the effects of sprouted durum wheat on pasta quality (Feillet, 1998).

Preharvest sprouting is known to influence the quality of durum wheat, but the intensity and nature of the damages are controversial. Increases in α-amylose and protease have been considered responsible for the change in pasta properties. By far α-amylose is the more harmful agent. From the recent works, following conclusions were obtained: α-Amylose activity induced a higher level of sugars, particularly maltose in spaghetti and subsequently increased the losses of solids in cooking water, with more simple sugars and HMW dextrin being leached (Feillet, 1998).

b) Lipoxygenases

Lipoxygenase refers to a group of enzymes that are highly specific for the oxidation by molecular oxygen of fatty acids (and lipids) such as linolenic and linoleic acids, to produce conjugated hydroperoxidiene derivatives.

It is generally well accepted that pigment loss on making spaghetti and subsequent yellowness of pasta are significantly correlated with lipoxygenase activities.

Under properly selected conditions of steam pressure and steaming time, the lipoxygenase activity of resulting semolina is reduced to zero, the yellow pigmnet of semolina being unchanged.

The role of lipoxygenase in pasta quality and the reaction mechanisms are not yet well elucidated. The possibility that semolina water-soluble fractions, particularly lipoxygenase, very effectively maintain the gluten at an unoxidized state, which is unfavorable to cooking quality has been suggested.

More studies are needed to provide better knowledge of durum wheat lipoxygenases in relation to overall oxidation of semolina components, including proteins, lipids, and other enzyme system (Feillet, 1998).

c) Peroxidases and Polyphenoloxidases

Peroxidase and polyphenoloxidase activities would control the browness of pasta product. A positive high correlation exists between the browness of macaroni and the peroxidase activity of semolina. Therefore it was recommended that to reduce browning of pasta, wheat cultivars with low peroxidase activity be selected (Feillet, 1988).

1.2.4. Other components

Fatty substances (lipids) are concentrated in the embryo of the wheat (germ), which moreover is removed before grinding to prevent the fats turning rancid and the semolina or flour rapidly deteriorating. However, there is always some vegetable fat in wheat milling products, above all in semolina, since eliminating the embryo in durum wheat less effective than for soft wheat. The presence of fats in the semolina helps decrease its preservation, but it also improves the technological characteristics of this raw material. During the formation of the dough, in fact, the fat component interacts in part with the protein, giving rise to lipoproteins whose role, as some scholars have noticed, has not yet been studied enough.
It is anyhow clear that the formation of lipidic bonds, both during the formation of the dough and during drying, reduces the stickiness of the pasta and its losses during cooking, since they limit the sugars (dextrins and amylose) on its surface (Martinella, 2004).

The presence of lipids however includes the drawback of their oxidation, so much greater if the semolina was preserved too long, since in this case the dough tends to absorb more atmospheric oxygen. The oxidation of the lipids (and the yellow pigments) of the semolina during the kneading phase can be avoided only with techniques that make it possible to prepare the dough without the presence of oxygen (in a vacuum). An alternative comprises the use of chemical anti-oxidants, such as for example L-ascorbic acid, whose use is moreover prohibited in Italy and the European Union in the production of dried pasta. Cellulose (lignin) and minerals are so much greater in semolinas and flours the higher the flour bolting rate, since their concentration is on the outside of the kernel and the germ, as regards the minerals. As concerns drying, these components have little importance, while they are important for the commercial value of the product, since too much of them has a negative effect on the appearance, even if it increases their biological value. The presence of minerals in the semolina affects in particular the amber-coloured appearance of the dried pasta (Martinella, 2004).

1.3. Continuous Manufacturing Process of Pasta

The current range of products referred to as pasta is relatively vast, even if these products vary widely in terms of shape, color, composition, storage requirements and use, etc. The aim of the pasta –making process is to transform a loose mass (semolina) into a mixture with a homogenous structure that is able to maintain a particular shape, which is then stabilized by drying (Dalbon *et al.*, 1996).

A greater or lesser number of variables are possible for each of the modules indicated in the diagram; in addition, this elementary diagram is normally enlarged or integrated with other process phases. As a result, extremely different types of pasta can be obtained despite the fact that the technological basis remains fundamentally the same. Practically speaking, the most common variables regard the nature and the composition of the raw materials used (flour, liquids, supplements and nutrients, etc.), the dough preparation and shaping techniques, and the other operations and respective processes performed on the product (Martinella, 2004).

A general classification of pasta that is valid for the international market defined on the basis of all the variables indicated (and their multiple combinations) is not easy, and somewhat meaningless, if the truth be told. It is more practical and useful to summarize the vast panorama of today's types of pasta schematically with regard to their physical state of conservation, commercial type, raw materials and ingredients used, the type of process used for product shaping and stabilization, and the manner of consumption (Martinella, 2004).

Pasta production technology has made enormous progress since the 50s, and further accelerated its pace of development in the pasta few decades through the stimulus provided by a more precise and complete scientific awareness of the product and the success of new models of consumption. The diffusion of pasta throughout the world and the popularity of both the traditional product and non-conventional products has provided and ulterior and tangible impulse to the development of production technologies and techniques (Martinella, 2004).

The fundamental steps in the technological process required to work transformation are:

- blending of flour and water (mixing) to form a homogenous mixture
- kneading of this mixture
- forming into final shape

These operations were originally the result of patient, skillful manual work. Development of the original construction has resulted in the modern continuous press; a machine, which uses increasingly, sophisticated technological arrangements to achieve a high hourly output and a final product of good quality (Martinella, 2004).

1.3.1. The Press

This unit shown in Figure 1.1 usually includes the following functional groups;

- raw material and ingredient doser
- mixing unit
- dough kneading machine
- extruder
- die

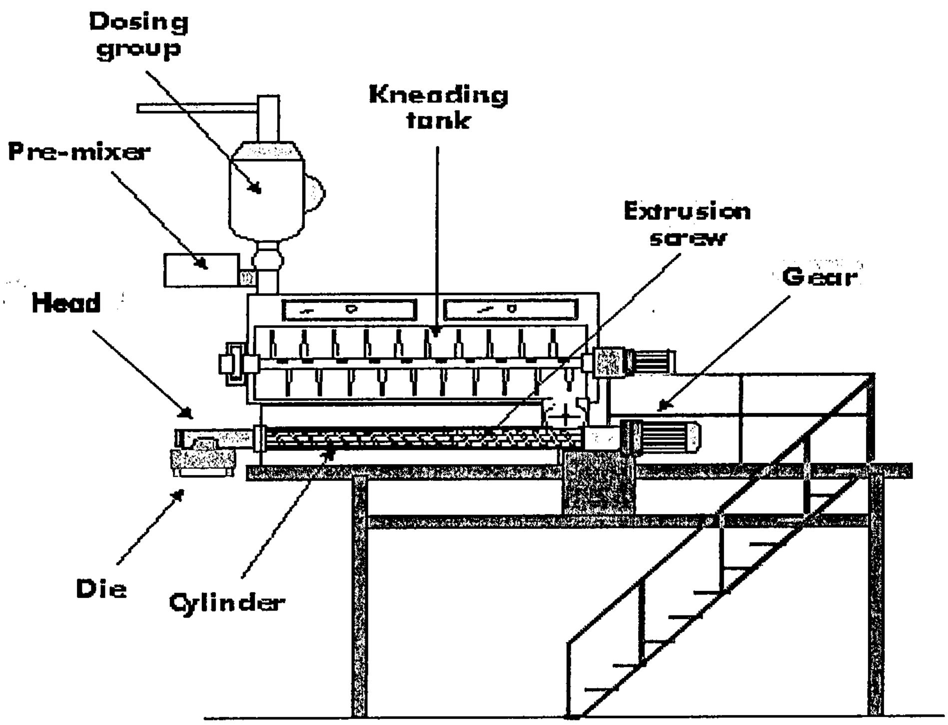

Figure 1.1. Layout of a modern continuous press (Martinella, 2004)

a) The raw material and ingredient doser

The aim of the this first group is to blend the prescribed proportions of the different ingredients making up the mixture (Dalbon *et al.*, 1996). Since pasta basic raw materials are flour (semolina or flour) and liquids (water), each dosing group is in fact made up of separate dosing units. Among the usually added ingredients, egg emulsion comes first, but other powdered products are used as well (dehydrated vegetables, food additives or powdered eggs). The dosing group has therefore to guarantee (besides precision) a steady synchronization between the different operating parts. Ingredients can be measured out referring to their volume (volumetric doser), to their weight (gravimetric doser) or in combined sequences. Predetermined ratio and semolina (about 30:100) must remain constant throughout the production cycle. In other words, any fluctuation in the proportions of water and semolina must remain within a narrow range of set values. If the proportions of ingredients do not remain constant, or if the set range of fluctuation is too wide, uniform distribution of the water among the flour particles never be obtained; even the subsequent kneading process will not be sufficient to produce a homogeneous dough (Dalbon *et al.*, 1996).

b) The mixer

The aim of the mixing is to distribute the water as evenly as possible throughout the semolina, thus promoting the hydration of particles. The amounts of water normally used in pasta-making produce a mixture with a granular texture. Mixing is carried out by the action of two mixing machines which operate in succession: the pre-mixer and the mixer.

The measured amounts of raw materials are fed into pre-mixer which serves to thoroughly mix the semolina, water and any other ingredients. The mechanical action of the pre-mixer promotes optimal distribution of the water throughout the semolina mass, thus shortening the time required for good preparation of the mixture (Dalbon *et al.*, 1996). It is very important that the solid (flours, powders) and the liquid (water, emulsions) are mixed thoroughly even before the dough making process. This operation is particularly critic for the flour particles (durum wheat semolina or soft wheat flour). If all the single particles don't absorb the liquids in the same way or don't reach the same degree of absorption all at the same time, it will hardly be possible to obtain a thoroughly homogeneous dough and the dry pasta will easily show more or less marked faults (white spots, for instance) (Martinella, 2004).

The flour/water mixture thus is delivered into the final premixer so that the mixture is conveyed by gravity (Dalbon *et al.*, 1996). In order to obtain an even and homogeneous absorption of the liquids (water and/or egg emulsion) by the flour particles (semolina and/or flour), at least two basic conditions have to be guaranteed:

1. the particles must have the same size or average size ranging between not too distant minimum and maximum values (granulometry);
2. the time needed for the liquid to be absorbed by the particles has to be evaluated while taking into account their average size and the temperature of both flour and liquid. The lower the flour temperature (semolina and flour), the higher has to be that of the liquids used in the dough (water and/or egg emulsion) (Martinella, 2004).

The rate of water absorption by individual granules depend upon the quality, type and particle size of the semolina and can vary considerably, causing "white spots" in the final product. These phenomena can be limited if the granulation is fine and above all homogeneous, and if semolina blends with a similar chemical composition are used (Dalbon *et al.*, 1996).

c) Extrusion

The final dough is extruded in the extrusion unit, consisting of a cylinder inside which a special extrusion screw turns. The screw rotation pushes the dough towards the head press on which a die is set. The pressure on the dough makes it go through the openings in the die and get the required product shape. The extrusion process allows continuous kneading and shaping of the dough to be carried out simultaneously and rapidly, in a matter of a few minutes. Extrusion is a key operation in the pasta making process as it determines both the rate of production and the quality of the end-product (Dalbon *et al.*, 1996).

d) Die

The die function is to give the product the chosen shape. The great variety of pasta kinds and sizes is due to the possibility of making specific dies. The long cut pasta, for instance, is produced in many different shapes that can be summarized, according to their section, as follows (Martinella, 2004):

- long round pasta without hole (e.g. spaghetti);
- long round pasta with hole (e.g. bucati, ziti, zitoni);
- long oval pasta (e.g. linguine);
- flat straight pasta with rectangular section (e.g. fettuccine);
- flat straight pasta with simple (on one side) or double (on both sides) festoons (e.g. mafalde, curly lasagna).

The dimensions of these shapes (section, diameter, length, width, thickness) can vary from a producer to another, even if many shapes have standard commercial values. The die is made of a bronze support with a shape which is usually rectangular for long cut pasta and round for the short one. In the bronze support (that must have the right thickness to give it the necessary strength for the high and continuous pressure it is subjected to), there are holes in which inserts are placed. The inserts are designed and patterned so as to give shape to the product. Both the die and the inserts have to be built with extreme precision and made of special materials (e.g. Teflon) able to assure not only the die life but also its efficiency, with concern to both the product quality (even surface, colour, etc.) and the press output capacity (Martinella, 2004).

The dies that are made entirely in bronze, directly holed by profiles suitable for the required shapes, are still used for rough surface pasta. Their average life is though considerably shorter than that of Teflon insert dies and are therefore used mainly for small special productions.

A die is a basic component of a press: the dough, formed in the kneading tank and then driven by the extrusion screw towards the head of the press, is forced through the die. This circular or rectangular device "shapes" the pasta. But its importance goes beyond this: the initial quality of the product is largely determined by the die, likewise the overall efficiency of the press. Making dies for a modern pasta factory requires many skills and great professionalism (Martinella, 2004).

f) Drying

Drying is the elimination of a liquid, normally water, from a substance or a solid body. The water to be eliminated may be just on the surface, or inside the solid body to be dried. If it is only on the surface, drying does not depend on the properties of the body at issue. Whereas, if the water is inside it, the methods of drying will depend on the body's physical and chemical properties. This is because the water particles need to move from the inside onto the surface in order to then be removed (normally by evaporation). Clearly, how this takes place depends on the characteristics of the body at issue (structure, nature, dimensions, type of chemical bond of the water molecules with the other components, etc.). And it is this last case that concerns drying pasta (Martinella, 2004).

1.4. Wheat Germ

1.4.1. Structure of the germ

The germ is a separate and distinct part of the wheat kernel. Partly embedded in the endosperm, it is located at the base of the wheat kernel. There are natural lines of separation of the germ from the endosperm and the bran. Thus, it would appear that the germ could be easily separated even from the dry kernel. Actually, the tissues of the germ and endosperm are in intimate contact with each other, probably through a cementing layer in between.

Structurally, the wheat kernel may be divided into three main parts: 1) the germ (the embryo), which produces new plant; 2) the endosperm which provides the food for the new plant when the embryo starts to grow; and 3) the varoius outer coverings, collectively called bran, which protects the kernel. The relationship of the different parts of the wheat kernel with special reference is given in Figure 1.2.

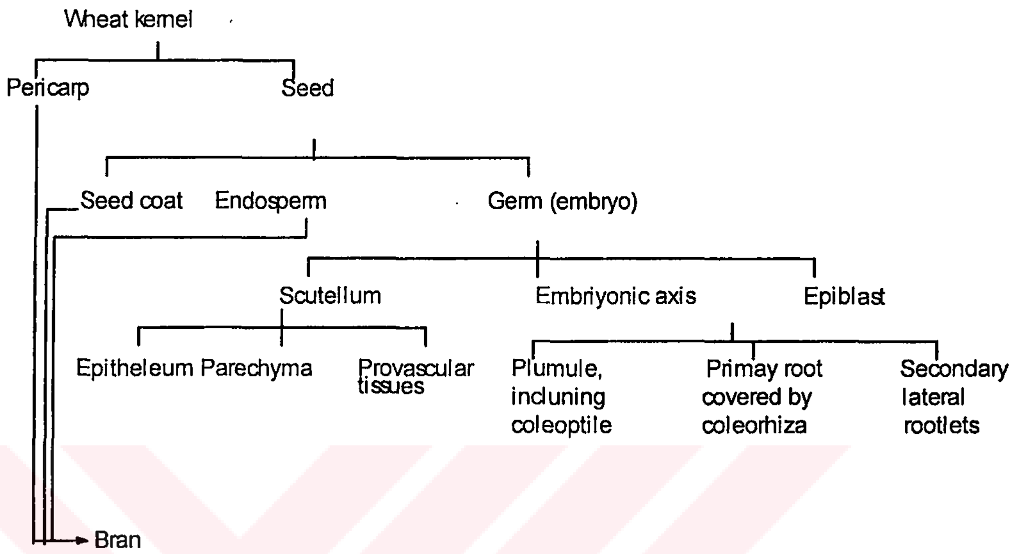

Figure 1.2. Wheat germ: structural parts (Shurpaleker and Rao Haridas, 1977)

1.4.2.Germ content and composition of its structural components

Drum wheat has a higher germ content, 2.94%, followed by soft wheat, 2.66%, and spring wheat, 2.33%. Recently it was observed that hard wheats contain a higher proportion of the germ (2.4 to 3.9%) than soft wheats (2.1 to 3.2%). Also, smaller kernels of any one variety have a smaller proportion of the germ than large kernels (Shurpaleker and Rao Haridas, 1977).

1.4.3.Chemical composition of the germ

The considerable variation observed in the proximate composition of mill germ due mainly to different degrees of contamination of bran as well as endosperm. The normal ranges of the proximate composition of commercial samples of mill germ are given in Table 1.1.

Table 1.1. Proximate composition of commercial wheat germ (Shurpaleker and Rao Haridas, 1977)

Constituent (%)	Range (%)
Moisture	9.0-13.0
Protein (Nx6.25)	18.3-35.3
Ether extractives	5.2-15.0
Mineral matter[1]	3.1-5.0
Cellulose (fiber)	1.8-4.0
Carbohydrate[2]	19.2-53.
Starch	14.0-23.9

[1]total ash
[2]by difference

Lower values of fat indicated the contamination of the germ with flour. The germs from hard red spring and drum wheat had appreciably higher fat and protein contents than did the germ from soft wheats. Carbohydrates present in commercial germ could be the best index of purity for most of the germ samples. The carbohydrates in germ fractions from hard red spring and drum wheats were the lowest (24.3%), compared with 30.4% for hard red winter, 36% for red white wheat, and 39% for soft red winter wheat. These observations indicated that the flour portion of the germ processed from hard wheats could be easily separated owing to its granular nature as compared with that of soft wheat germ.

The comparative proximate composition of commercial samples of flour, germ, and bran are shown in Table 1.2. The data indicated that the germ contains three times as much protein, seven times as much fat, six times as much ash, and about fifteen times as much sugar as does endosperm flour. Unlike others, these workers have reported the carbohydrate values for different fractions individually and not by difference (Shurpaleker and Rao Haridas, 1977).

Table 1.2. Proximate composition of commercial mill products (Shurpaleker and Rao Haridas, 1977)

Composition	Endosperm (%)	Germ (%)	Bran (%)
Moisture	14.7	12.6	13.2
Protein (Nx5.7)	11.3	29.8	14.4
Fat	1.4	10.6	4.7
Ash	0.7	4.7	6.3
Carbohydrate (by difference)	74.3	44.9	66.3
Starch	71.0	14.0	8.6
Hemicellulose	1.8	6.8	26.2
Sugar	1.1	16.2	4.6
Cellulose	0.2	7.5	21.4
Total carbohydrate	74.1	44.5	60.8
Recovery of fraction	99.8	99.0	99.4

To summarize, there is considerable variation in the literature values reported for the proximate composition of the germ and its fractions. However, the values reported recently by study1 in Table 1 for germ milled from nine varieties of wheat can be taken as the best representative values for normal samples (Shurpaleker and Rao Haridas, 1977).

a) Germ protein

The factor 6.25, customarily applied to convert the total nitrogen figure to protein content, is considered to be too high, and recommended factor for germ protein is 5.45. This is based on the amino acid content of the germ and reflects the substantial amount of non protein N present. The protein content of mill germ (factor 6.25) at 14% moisture was reported as 17.7-35%, the wide range probably reflecting the amount of contamination. The values 21.4-25.6%, as the range for nine wheats, are probably the best representative figures for normal mill samples. Germ protein types, as percentages of total, are albumin, 30.2%; globulin, 18.9%; gliadin, 14%; glutenin, 0.3-0.37%; and insoluble types, 30.2%.

Reported amino acid contents of germ and germ protein are given in Table 1.3. There is a good agreement between data in columns A and B, which were obtained by different methods of determination.

Table 1.3. Amino acid content of mill germ and of germ protein (Pomeranz, 1988)

Amino Acid	In Germ, 14% Moisture	In Protein
Alanine	1.34	
Arginine	1.77	6.2
Aspartic acid	1.92	
Cystine	0.43	1.37
Glutamic acid	3.65	
Glycine	1.32	
Histidine	0.59	3.03
Isoleucine	0.77	5.23
Leucine	1.5	7.33
Lysine	1.3	5.44
Methionine	0.39	1.28
Phenylalanine	0.86	4.2
Proline	1.13	
Serine	1.05	
Threonine	0.89	6.28
Tryptophan	2.44	0.9
Tyrosine	0.65	
Valine	1.68	4.2

The non protein nitrogen content is 11.3-15.3%. It includes the following, in amounts expressed as proportions of germ (14% mb): asparagine, allantoin (total amide N, 0.53%), 0.7%; betaine, 604-3.062 ppm; choline, 0.30-0.39%; lecithin, 1.25%; and glutathione, 0.345-0.460% (Pomeranz, 1988).

b) Germ lipids

Wheat germ contains a high percentage of fat compared with other mill products like bran, patent flour, and shorts. Common values for wheat germ oil are following: refractive index, 1.4700-1.4800; specific gravity, 0.900-0.9300; iodine value, 184-185; free fatty acids, up to 6%; and unsaponifiable matter which contained sterols, tocopherols, and pigments, 1.5-7.8%. Because of the high iodine value, germ oil was classified among the semidrying oils. It was shown that 74% of lipids of germ and bran were nonpolar (tri-, di-, and fatty acids, steorols) and less than 25% consisted of polar lipids (phospho- and glycolipids). The major fatty acid, linoleic acid, accounts for about 60% of the total; combined fatty acids are at least 80%; most of the saturated acids is palmitic acid; and stearic acid is below 2%. Wheat germ has high amount of the unsaturated fatty acids (Table 1.4).

Table 1.4. Unsaturated fatty acid composition of wheat germ

Fatty acid	Composition (%)
Total unsaturated fatty acids	84.00
α-Linolenic acid	1.83
β-Linolenic acid	1.72
α-Linoleic acid	22.32
β-Linoleic acid	29.99
Oleic acid (by difference)	28.14

Palmitic acid, the principal fatty acid, formed 73.5% of the total saturated fatty acids. However another study reported a higher value of 91%. The remaining 9% was mostly stearic and lignoceric (Pomeranz, 1988; Shurpaleker and Rao Haridas 1977).

Fatty acid composition of wheat gem oil was compared with fatty acid composition of some oils by looking literature values, and summurised in Table 1.5. It was found that fatty acid composition of wheat germ oil was not same but similar with soybean oil.

Table 1.5. Comparison of fatty acid composition of wheat germ oil with some other oils

Fatty acid composition	Wheat germ[1]	Soybean[2]	Corn[3]	Conola[3]	Olive[3]	Sun Flower[3]	Sesame[4]
Palmitic (C16:0)	14.56	11	10.7	4.7	12.9	6.2	8.02
Stearic (C18:0)	1.44	2	1.6	2	2	3.5	5.38
Oleic (C18:1)	28.14	21	24.5	60	71.2	20.7	39.59
Linoleic (C18:2)	52.31	55	61.3	21.7	10.7	67.9	45.66
Linolenic (C18:3)	5.2	6	1.1	9.1	0.9	0.2	0.3

[1]Shurpalekar and Haridas Rao, 1977; [2]Luzia *et al.*, 1998; [3]Chu and Kung, 1998
[4]Abuo-Gharbia *et al.*, 2000.

During 30 days of storage of laboratory extracted oil in the dark, it was observed that the acidity index of the oil increased from 5.27 to 17.82 and the peroxide index from 0 to 0.08 at ambient temperature, but remained unchanged at 4°C. Further it was observed that resistance of germ oil to autoxidation was double (12.7 hours) that of sunflower oil (6.2 hours) in a modified Schaal oven test at 100 °C (Shurpaleker and Rao Haridas, 1977).

c) Germ carbohydrates

Generally, the carbohydrate content of any food is calculated by difference. The carbohydrate content of mill germ varies widely (19.2 to 53.0%; see Table 1.1), depending on the contamination with the endosperm and bran portions. It is therefore, the best index of purity of mill germ.

Hemicellulose, cellulose, starch and sugars were found as the carbohydrate fractions of mill germ. Sugars formed the major component of the germ, while endosperm was rich in starch, and bran was rich in cellulose and hemicellulose. The starch in germ was contributed entirely by endosperm impurity, while the cellulose and hemicellulose came from the bran present in the germ. The germ cellulose was made up of 52.1% xylose, 40.9% arabinose, and 7.0% uronic acid.

Using 80% hot alcohol for extraction, 15 to 18% of total sugars in deffatted germ was found. Of this, 80 to 90% was sucrose. The rest consisting of nonfermentable, nonreducing sugars (before hydrolysis), was strongly dextrorotatory and was hydrolyzed by yeast invertase to reducing substances. These characteristics resembled those of raffinose. 24.34% of total soluble carbohydrates were reported. Of these, 6.89% was raffinose, and the remaining 17.4 was persumed to be sucrose (Shurpaleker and Rao Haridas, 1977).

d) Germ vitamins

Wheat germ contains more tocopherol (vitamin E) than any other cereal germ. Mill germ contained 250 to 500 ppm of tocopherols with an average of 332 ppm. The germ contained five times as much thiamine as did whole wheat and about twenty-five times as much as did patent flour. Similarly, values for riboflavin in the germ were about four and seven times as high as those in wheat kernel and patent flour, respectively. Also it was reported that wheat germ contains niacin, pantothenic acid and pyridoxine (Shurpaleker and Rao Haridas, 1977).

e) Germ minerals

Minerals and contents are shown in Table 1.6.

Table 1.6. Mineral elements in wheat germ (14% mb) (Pomeranz, 1988)

Element	%
Potassium	0.47-1.3
Magnesium	0.14-0.36
Calcium	0.037-0.071
Phosphorous	0.8-1.24(av. 1.03 with 42-52% phytate P) ppm
Sodium	23.2; 130-340; 3.440
Manganese	1-232 (av. 93)
Zinc	100-140
Molybdenum	0.67
Selenium	0.01-0.77
Aluminum	5-7.4; 2.150
Nickel	40
Lead	1.1-1.8
Silver	0.5-1.3
Iron	39-240 (av. 94)
Copper	7.2-11.8; 775-3.960
Cobalt	0.09-0.017
Strontium	0.5-3.6
Boron	3.0-6.4
Barium	3.4-9.8
Tin	0.3-0.5
Silicon	90

f) Pigments

Apart from traces of carotenes, it was found that xanthophylls are the main pigments of the germ. Flavone-type pigments were reported to be present in greater proportions than in the other mill fractions. About 0.2 to 0.3% of glycoflavones were isolated from wheat germ (Shurpaleker and Rao Haridas, 1977).

g) Other organic compounds

Phenolic compounds such as phenolic, ferulic, and vanillic acids were detected in commercial germ. It was reported that antioxidant properties of wheat germ oil and attributed it to the presence of caffeine and ferulic acid compounds. Methoxyhydroquinone glycosides in the germ was detected and found to be mostly concentrated in the germ. 0.1% oxalic acid and 36.59 to 41.04 μmoles per gram thiochtic acid content in wheat germ was reported (Shurpaleker and Rao Haridas, 1977).

h) Enzymes

Lipase is probably the most important enzyme studied. This may be attributed to the major role played by lipase in the development of hydrolytic rancidity during storage of germ or products containing germ. Inactivation of lipase assumes paramount importance for improving the shelf life of the germ and germ products. Wheat germ lipase had an optimum temperature of 36 to 40°C and an optimum pH of 7.2 to 7.9 for esters of low-as well as high-molecular weight acids like butter fat and monobutyrin.

Amylases, proteases, phosphatases, oxidases (lipoxidase), catalase that play a part in the unsaturated fat oxidase system, peroxidase are the other enzymes present in the wheat germ (Shurpaleker and Rao Haridas, 1977).

1.4.4. Storage and stabilisation of the germ

Raw wheat germ was found to have a "feedy" taste and smell and poor stability owing to hydrolytic and oxidative changes in the germ lipids because of high enzyme activity and unsaturated nature of fat present. These defects have been overcome by heat processing of wheat germ to make it suitable for human consumption and to improve its keeping quality (Shurpaleker and Rao Haridas, 1977; Rao *et al.*, 1980; Rand and Collins, 1958; Ferrara *et al.*, 1989).

It was observed that increase in the germ acidity was a function of temperature and moisture. The acidity of the germ stored at 12.3% moisture was higher than that of the germ stored at 4.3%. Rancidity and peroxide values in germ oil increased concurrently during storage, whereas in whole germ the rancidity was detectable even when the peroxide values were relatively low. As such, the peroxide value was found to be better index of spoilage for the oil than for germ. It was therefore suspected that protein may be mainly responsible for spoilage of the germ, rather than lipids. It was reported that microorganisms did not play a decisive role in the development of rancidity in the germ. It was observed that in stored germ an increased lipoxidase activity which was dependent on the temperature of storage. According to studies, both lipase and lipoxidase activities in the germ were responsible for the development of off-flavors during storage. Stability of germ was increased by reducing the lipase and lipoxidase activities. It was reported that oxidation of fat was the main reason for the development of off-flavors in the germ. According to study, fat is converted to hydroperoxides either by autoxidation or by an enzymic reaction mainly by lipoxidase. Normally lipoxidase reaction was faster than autoxidation. The hydroperoxides could be further oxidized to polymerized compounds, which impart a bitter taste, or to carbonyl compounds, which give rancid taste to stored germ (Shurpaleker and Rao Haridas, 1977).

1.4.5. Methods of stabilisation of wheat germ

Raw germ on storage develops a rancid flavor and bitter taste in a short time. It was observed that, because of the high enzyme activity and unsaturated fat content in fresh germ, the organoleptic acceptability is affected adversely within a few days.

Autoxidation is considered to be main route of spoilage of edible oils, with their high proportion of unsaturated fatty acids, has the potential to develop undesirable odors and flavors during storage. The flavor of oxidized oils is attributed to primary and secondary oxidation products. The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxide (Vieira and Regitano d'Arce, 2001). Hydroperoxides are odorless themselves. Their decomposition leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the stale flavor of foods and may also be involved in biological oxidation (Abuo *et al.,* 2000). The presence of antioxidants and the polyunsaturated fatty acid (PUFA: C18:2 and C18:3) content are important in affecting the oxidative stability of oils (Chu and Kung, 1998). The extent of oil oxidation has been frequently evaluated by measuring peroxide values (Yaghmur *et al,*. 2001). The less amount of PV was preferred due to greater freshness. Acid value was another parameter related with oil quality. High acid value is not accepted in any commercial product because of the strong off-flavor caused by degradation products of the free fatty acids (Yaghmur *et al.,* 2001).

The poor stability of raw germ has restricted its food uses; this problem could be overcome either by inactivating enzymes or by creating conditions unfavorable for ezymic activity by suitable means. Some of the important methods for stabilisation of the germ and the effect of different treatments on the enzyme activities are steaming and toasting. Microwave treatment is new method used for stabilisation of wheat germ.

Effect of microwave treatment on the keeping characteristics of wheat germ was studied by Zwingelberg and Fretzdorff (1996). They found that microwave treatment improved shelf life of wheat germ up to 1 year without loss of nutritive value. Initial moisture contents of 11.5 or 14.2% were treated in a microwave with varying critical parameters; microwave energy (1.5-3.3 kW), layer thickness (15 to 30 mm) and temperature (70-135°C). Results indicated that small quantities of wheat germ in 7-8 mm layers could be satisfactorily treated by microwaves without damaging the enzymes, tocopherols or other heat-sensitive constituents, eliminating both the need for intermediate storage and the bitterness usually associated with other methods (Zwingelberg and Fretzdorff, 1996).

Light toasting of the germ at 120°C to 130 °C, until it attained a light brown color, improved its keeping quality as well as its palatability. When stored in paper cereal cartons at room temperature, the toasted germ was in good condition even after 10 months of storage.

The effect of heating germ, at various moisture levels for different periods in a hot air oven, on the enzyme activities and its stability during storage at 37°C in laminated metal was studied. It was found that all the heat treatments completely destroyed lipoxidase activity; the proteolytic activities were reduced considerably, depending on the moisture level.

The other method of stabilisation developed was direct steaming of the germ in a chamber at 110 °C for 30 minutes and then drying it at 100°C under nitrogen (Shurpaleker and Rao Haridas, 1977).

Rao *et al.* (1980) used defatting, drum (roller) drying, moist and dry heat treatment for stabilisation of wheat germ at different time and temperatures. The results indicated that the time required for optimum toasting of germ in an air circulation oven decreased drastically from 175 to 17 minutes, when the temperature of heat treatment was raised from 110 to 160°C. The germ toasted at 150°C for 25 minutes had an attractive light brown colour, pleasing aroma and improved sweetish taste. It was also observed that the toasting of germ at 120 to 130°C, to a light brown colour resulted in an improvement in its palatability as well keeping quality. It is a well established fact that moist heat treatment is more effective than dry heat treatment in inactivating the enzymes. They found that steaming of germ spread into layers of 2.5 cm thickness in solid bottom trays at atmospheric pressure for 10 minutes was found to be adequate for total activation of lipase. Subsequently they found that steaming at atmospheric pressure for 10 minutes in a horizontal autoclave at 5 cm thickness were stabilised germ successfully with activation of lipase totally. For drum-drying of wheat germ on steam heated twin rollers rotating at 4 revolution per minute they decided that slurry containing 33% germ solids was optimum for at a steam pressure of 35 psi, corresponding to a drum temperature of 138°C.

As a result it was found that the increase in FFA and PV of defatted and drum-dried germ samples were exceptionally higher, as compared to those of toasted or steamed germ. The toasted and steamed germ samples stored at 37°C were acceptable even at the end of 26 weeks. The drum-dried sample kept well for 20 weeks, after which development of a slight off-flavour was observed. The defatted germ behaved somewhat differently from the heat processed germ samples and was found acceptable only for a period of 12 weeks, whereafter development of bitterness was observed (Rao *et al*., 1980).

In studying the nutritive value of raw germ meal for chick, it was found that a thermolabile factor which impaired digestion and/or utilization; autoclaving significantly improved growth and feed efficiency. Further investigation revealed that autoclaving whole raw wheat germ also significantly increased nitrogen retention and fat utilization by the chick. It was confirmed previously observed growth depression and increased fecal fat; however, it was contended that the adverse effects of raw meal were physical in origin.

Processed whole germ is preferred to raw germ for feeding, since raw germ depress growth and fat utilization. The depression was eliminated when germ was steamed or autoclaved. Raw germ contains deletorious hemagglutination and antitrypsin factors. Moran and Summers (1967) recently reviewed the effects of toasting and autoclaving wheat germ meal (fed as a sole source of diatery protein) on utilization and protein quality for the growing chick. Both toasting and autoclaving destroyed hemaglutination and antitrypsin activities. It was found no toxic components in raw wheat germ but reported that the high nutritional value was reduced by heat-treatment. However, toasting significantly improved net protein utilization values (NPU), while autoclaving had no apparent effect. Prolonged autoclaving destroyed lysine, cystine, and arginine, reduced NPU, and seriously impaired chick growth and feed utilization (Pomeranz *et al.*, 1970; Pomeranz, 1988).

Heat-processing may not only improve the value of feedstuffs but, if excessive, may reduce their effectiveness. The browning or maillard reaction is known to occur between various free sugars and the free amino, quanido, and imidazole groups of proteins, resulting in nutritionally unavailable complexes. Temperature, moisture duriation, possibly reducing chemicals, e.g. sulfites, and availibility of free sugars are the prime factors among the many considerations which can affect the rate of reaction. Lysine is amino acid primarily affected by the browning reaction regardless of protein source. Lysine is also the most limiting amino acid for low-income humans, not to mention monogastrics of economic importance. Wheat germ protein is high in lysine. Consequently, processing of the germ must be sufficient to ensure utilization of inherent nutrients, but no so extensive as to detract from its prime asset, lysine.

Moran and Summers (1967) found that the toasting procedure of processing wheat germ meal always proved nutritionally better than any autoclaving treatment. The observed differences in quality can be related back to one outstanding difference in processing-the presence of moisture. The resultant low moisture content of germ meal with toasting and its removal from system upon evaporation are probably the reasons for its superiority over the 20-min. autoclaving treatment. This superiority was particularly true when either protein was critical (15%) or sulfur amino acids low, or both. With denaturation of raw wheat germ protein, one would expect increased digestibility because of decreased resistance to enzymatic degradation and destruction of toxic factor(s). During denaturation, destruction of cystine by cross-link cleavage is an expense of the process. It would appear that denaturation was sufficiently extensive to facilitate digestion, but not so complete as to detract from the meal by destroying its first limiting amino acid, i.e., hydrogen bond disruption but not cross-link cleavage. The cystine content of protein is known to be affected by heat-treatment regardless of the presence of glucose or extent of browning. On the basis of results of this experiments, it is more likely that cystine destruction is an indication of denaturation of proteins containing low or moderate quantities of this disufide amino acid and is not necessarily a desirable phenomenon.

Moisture content may also affect destruction of lysine and arginine, and hence, protein quality. The presence of moisture probably facilitates the browning reaction by acting as a carrier for the free sugar(s) and as a mode by which reaction ensures. Because of low concentrations of free monosaccharides *per se*, moisture could also facilitate the availibility of substrate(s) by entering directly into hydrolytic reactions with more complex carbohydrates such as starch and dextrin. The reduction of browning with large quantities of water, apparently by preventing direct exposure, supports the view that the browning reaction is, in part, oxidative , as does inhibition by sulfites, and suggests another possible method of processing (Moran and Summers, 1967).

1.5. The aim of the thesis

The aim of the thesis was to investigate shelf life of wheat germ and enrich macaroni with known shelf life properties of wheat germ.

In this study macaroni enriched with wheat germ was not intended to replace regular macaroni product, but it was produced to provide a nutritious and high protein containing food.

Wheat germ was selected for enrichment of macaroni because of its relatively high protein content, abundance of vitamins and ready availability and also it has a valuable and economic human feedstuff. The germ protein, though of plant origin, is reported to have nutritive value comparable to that of animal proteins, which are highly expensive. Apart from its protein, wheat germ is an excellent source of tocopherols, B group vitamis and oil, made up of a relatively large proportion of unsaturated fatty acids.

Wheat germ was a by-product of flour milling industry and in our country it is generally used for animal meal. The reason may be the properties of raw wheat germ that was found to have a "feedy" taste and smell and poor stability owing to hydrolytic and oxidative changes in the germ lipids because of high enzyme activity and unsaturated nature of fat present.

In this study, enriched macaroni samples were produced by mixing 85% semolina with 15 % raw or microwaved wheat germ. Microwave heating was done to overcome poor stability of wheat germ. The samples were stored one year at room temperature and analyzed for sensory attributes, cooking quality, color, compressibility, microbial count, *in-vitro* protein digestibility and gelatinization phenomena of starch during storage. Results were compared with results of control macaroni.

CHAPTER II

MATERIAL and METHODS

2.1. Materials

Wheat germ was obtained from a commercial wheat flour factory in Gaziantep, Turkey.

Glacial acetic acid, diethyl ether, 2-thiobarbutiric acid, potato dextrose agar, chloroform, cupper (II) sulfate pent hydrate were obtained from Merck (Dermstadt, Germany). 1,1,3,3-tetraethoxypropane(TEP) and trypsin were obtained from Sigma (St. Louis, Mo). Sodium thiosulfate –5 hydrate, phenolphthalein, sodium hydroxide, starch, boric acid, ammonium thocyanat, hydrochloric acid (37%) were obtained from Reidel De Haen (Germany). Potassium chloride from Carlo Erba (Italy) and total plate count agar from Acumedia (Omaha, Nebraska) were used. All these chemicals were analytical grade. Chloroform and methanol used for extraction of wheat germ were technical grade.

2.2. Methods

2.2.1. Shelf-life studies of whole wheat germ and wheat germ oil

2.2.1.1. Extraction of oil from wheat germ

A 250 g wheat germ sample was weighed into a 2 L beaker. A 750 ml mixture of chloroform:methanol; 2:1 (v/v) (Malecka, 2002; Erickson, 1993) were added and mixed for 2 hr and after filtration solvent was evaporated using rotary vacuum evaporator. The oil obtained was stored at –20 °C, in the dark until used.

2.2.1.2. Shelf-life studies

a) Wheat germ oil;

Wheat germ oil which was stored at –20 °C was defrosted at ambient temperature (approximately for 1.5 hr). Then 10 ml sample was placed into test tubes, covered with aluminum foil and placed in to ovens at 30 and 40 °C. During one mounth storage period, free fatty acid (FFA), peroxide value (PV) and thiobarbutiric acid (TBA) test were performed to determine shelf-life of wheat germ oil.

b) Whole wheat germ;

Whole wheat germ samples were placed in closed plastic bags and stored at 30, 40, 60, 70 and 80 °C in ovens. During 2.5 month storage 250 g sample was taken from oven and oil was extracted as explained above. Extracted oil samples were analyzed to determine FFA and PV.

2.2.2. Production of macaroni

2.2.2.1. Microwave treatment of wheat germ sample

A 250 g wheat germ sample was processed at 100% power level for 12 min with intermittent mixing using a 700 watt microwave oven (Arçelik, 2450 MHz). The final temperature of the sample was approximately 110 °C. Treatment of wheat germ at these microwave conditions is reported to be sufficient enough to deactivate enzyme activity (Zwingelberg & Fretzdorff, 1996). During microwave treatment the samples were stirred manually after every 3 min heating, due to local burning of the samples.

2.2.2.2. Preparation of semolina and wheat germ blends

Raw or microwaved wheat germ which were milled to semolina size (132-145 μm) was added to durum semolina at 15% (w/w) level and mixed to obtain a homogeneous mixture by using a paddle type mixer.

2.2.2.3. Macaroni production and storage

Macaroni was produced in a commercial macaroni production factory. It was prepared using a short cutting line of the factory with a capacity of 700 kg/hr. In this study, 100 kg semolina was used for production of control macaroni and 15 kg raw wheat germ or 15 kg microwaved wheat germ was mixed with 85 kg semolina for production of raw and microwaved wheat germ added macaroni respectively. Moisture content of macaroni was 30, 19 and 12% (w/w, db) after exit from pre drying (40 °C), softening (55-60 °C) and final drying (60-65 °C) sections, respectively.

a) Preliminary work

Optimum wheat germ percentage for macaroni production was determined by using raw wheat germ at a rate of 5, 10, 15 and 20%. Produced macaroni samples were analyzed for ash, soluble solid, moisture and protein content. The results were compared with food codex (Anonymous, 2002). 15% wheat germ addition was found as optimum for macaroni production.

b) Macaroni production

Macaroni was produced with addition of 15% raw and microwaved wheat germ into semolina. The 500 g samples were packaged in polypropylene bags, stored in a dark place at room temperature and analyzed immediately, after 6 and 12 months of storage.

2.2.3. Chemical analysis

2.2.3.1. Determination of protein content

Kjeldahl method (AOAC, 1990) was used to determine crude protein of wheat germ and macaroni samples through digestion, distillation and titration steps. For digestion 2 g milled sample, 10 g potassium sulfate, 25 ml sulfuric acid, small amount of copper sulfate and boiling chips were put into the digestion tube and heated until digestion was completed (nearly 2 hr). When the digestion was completed, clear light green color was observed.

For distillation, 50 ml water, 50 ml of 50% sodıım hydroxide and steam was used and distillate was collected into erlenmayer flask, which has 25 ml of 4% boric acid.

For titration, methyl red was added as indicator to the flask, which contains distillate and boric acid, and titration was done by 0.1 N hydrochloric acid. From the titration volume, the amount of N-NH_4 was calculated and protein content was found by multiplying this value by 5.7.

2.2.3.2. Determination of fat content

Crude fat determination of wheat germ and macaroni samples were performed by extracting samples with hexane for 6 h (AOAC, 1990). Hexane was removed from oil by using rotary vacuum evaporator.

2.2.3.3. Determination of ash and moisture content

Ash and moisture content of wheat germ and macaroni samples were determined according to ICC No: 110 and 104 respectively (Anonymous, 1960).

Ash content of samples was determined in Nüve MF 120 type oven. 5 g milled sample was mixed with 2 ml ethanol and ignited at 900 °C until the cooled sample was white or nearly white. From remaining sample ash content was calculated.

Moisture content of samples was determined by heating 5 g milled sample at 130 °C for 2 hr in the oven.

2.2.3.4. Determination of free fatty acid (FFA) and peroxide value (PV)

FFA content of wheat germ oil was determined by titration method according to Pearson (Pearson, 1976).

5 ml of diethyl ether was mixed with 5 ml alcohol (95%) and 0.2 ml phenolphthalein. Mixture was neutralized carefully with 0.01 N sodium hydroxide (NaOH). Later 1 g of the oil sample was mixed with neutralized mixture and titrated with 0.01 N NaOH until pink color, which persists for 15 sec was obtained. Then spend volume of NaOH was used for calculation of FFA %.

PV of wheat germ oil was determined by titration method according to AOAC methods (1990).

5 g oil sample was mixed with 1 ml saturated potassium iodide and stirred vigorously for 1 minute. Then 25 ml of acetic acid/chloroform mixture (3/2) was added and this mixture was waited for 5 minute in a dark place. Later 75 ml of distilled water and 1 ml starch (1%) solution was added and mixture was titrated with 0.01 N sodium thiosulfate and volume was recorded. Also blank solution was prepared by using 5 ml water instead of 5 g oil and volume was recorded. Peroxide value was calculated by using these volumes.

2.2.3.5. Determination of thiobarbituric acid (TBA) value

Malonaldehyde which is secondary product of lipid oxidation was determined by TBA value. Malonaldehyde react with TBA and forms colored complex (Addis, 1986).

TBA value of wheat germ oil was determined by spectrophotometric method at 538 nm by using TBA reagent described by Tarladgis (1960).

2.2.3.6. Determination of cooking quality of macaroni samples

Percent cooking loss , weight and volume increase were determined according to method described by Olfat *et al.*, (1993). Approximately 10 g sample of macaroni was placed into 300 ml of boiling distilled water in a 500 ml beaker. After simmering for 10, 20 and 30 min, samples were washed through with distilled water and allowed to drain for 2 min. Drained macaroni samples were analyzed for their cooking loss, weight and volume increase. Cooking water of the samples was collected in a beaker and dried in a 130 °C oven until constant weight to determine cooking loss. Weight increase of macaroni samples were measured by using sensitive balance and then they were placed in to 250 ml measure to determine their volume increase. Cooking loss, weight and volume increase was calculated by the following equations:

Weight increase (%) = [(Weight of cooked macaroni – Weight of uncooked macaroni)/Weight of uncooked macaroni]*100 **(2.1)**

Cooking loss (%) = [(Weight of residue in cooking water)/ Weight of uncooked macaroni]*100 **(2.2)**

Volume increase (%) =[(Volume of cooked macaroni – volume of uncooked macaroni)/volume of uncooked macaroni]*100 **(2.3)**

2.2.3.7. Color

Color of macaroni was measured with a Hunter Lab solorimeter (Colorflex, USA). Before each of the color measurement, white standard tile was used to calibrate colorimeter (L=91.08, a=-1.14, b=1.31). Duplicate measurements for L, a, and b were done. Measurements as L, a and b values were standardized with a white color standard where L is the brightness coefficient from dark (0) to bright (∞), a the coefficient from green (-) to red (+), and b the coefficient from blue (-) to yellow (+).

2.2.3.8. Acidity of cooking water of macaroni

Acidity of cooking water was measured by titration method. 10 ml cooled cooking water sample was titrated with 0.01 N NaOH after addition of phenolphtalein as an indicator. Acidity was given in terms of percent lactic acid (LA%) (AOAC, 1990).

2.2.3.9. Texture analysis

Cooked products were drained for 1 min and a compressibility test was performed using a TA.XT2i texture analyzer (Texture Technologies Corp, Scorsdale, NY/Stable Micro Systems, Surrey, UK) within 15 min of cooking. The compressibility of cooked product was determined using a modified method of Matsuo and Irvine (1971) and Wang, Bhirud, Sosulski and Tylor (1999). Compression of macaroni samples was expressed as the time required to reach 50 g-force exerted by the probe. Compression data of each sample were obtained after 10, 20 and 30 min cooking. Results were the average of triplicate determinations. The probe had rectangular contact surface which has the dimension of 5 cm legth, 3.7 cm witdh and 0.9 cm thickness. The instrument was equipped with a 25 kg load cell and operated with following parameters: a) loading speed prior to sample contact, 2.5 mm/s; b) trigger point, 5.0 g-force; c) loading speed during compression of samples, 0.5 mm/s; d) maximum compression force, 50 g-force; f) loading speed during retraction and sensing of compression, 5 mm/s.

2.2.3.10. *In-vitro* protein digestibility

In-vitro protein digestibility of uncooked macaroni samples were determined by modified methods used by Hsu, Vavak, Satterlee and Miller (1977) and Dahlin and Lorenz (1993). The samples were ground to a fine powder that was able to pass through 80 mesh screen. Distilled water was used in preparing all solutions. Fifty milliliters of aqueous protein suspension based on crude protein content (6.25 mg protein/ml) were allowed to rehydrate for 60 min at 5 °C with intermittent mixing. After rehydration samples were placed in a 37 °C water bath and pH was adjusted to 8 using 0.1 N NaOH and/or 0.1 N HCl, while stirring. Lyophilized, crystallized trypsin (Sigma Chemical Co., St Louis, mo) at a concentration of 1.6 mg/ml maintained in an ice bath and pH was adjusted to 8 with 0.1 N HCl and/or 0.1 N NaOH. Five milliliters of enzyme solution was then added to the protein suspension, which was being stirred at 37 °C. The trypsin had an activity of 13766 BAEE units/mg protein. A rapid decline in pH occurred immediately. The pH drop was recorded at 15 s after enzyme addition followed by one min intervals for ten min. Triplicate analysis was performed for each sample. The enzyme solution was freshly prepared before each series of tests. The percent protein digestibility (Y) was calculated from the equation 2.4 (Hsu, Vavak, Satterlee & Miller, 1977),

$$Y = 210.464 - 18.1x \quad \textbf{(2.4)}$$

where x is the pH change after 10 min.

2.2.3.11. Differential scanning calorimetry

Ground macaroni, semolina, raw and microwaved wheat germ samples were sieved to pass through 425μm mesh screen. Two different blends of semolina were prepared by mixing with 15% raw and microwaved wheat germ. Prepared semolina blends, semolina and macaroni samples were weighed (12 mg) in to differential scanning calorimetry (DSC) pans. The samples were wetted with 36 μl distilled water carefully with micropipette, using ratio of dry sample to water at 1:3 (Güler, Köksel & Ng, 2002; Villwock, Eliasson, Silveria & BeMiller, 1999; Biliaderis, Maurice & Vose, 1980). DSC pans were sealed and allowed to equilibrate in a refrigerator at 1.5 hr prior to analysis. After a trial, the equilibration (resting) times for the mixed sample was determined to be 1.5 hr, since no difference was noticed between 1.5 hr and 24 hr resting time. This clearly showed that, a homogeneous hydration would be obtained after 1.5 hr of waiting period.

Samples were analyzed triplicate with Perkin-Elmer DSC 6 (Perkin Elmer Ltd, Norwalk, CT). The instrument was calibrated with indium, and empty pan was used as reference. The samples were heated at a rate of 4 °C/min from 4 to 140 °C with nitrogen flushing (40 cm^3/min). The instrument sensitivity was 0.5 mcal/s (Zweifel, Cande-Petit & Escher, 2000).

The starch gelatinization characteristics in DSC thermogram can be explained by various temperatures. For each endotherm the onset temperature (To), the conclusion temperature (Tc), the peak temperature (Tp) and the enthalpy (ΔH) was computed automatically by using software program. Furthermore, melting ranges of each peak (ΔT=Tc-To) were calculated.

2.2.4. Sensory evaluation

Two types of sensory evaluation were performed.

a) Triangle test

Triangle test was used as sensory evaluation method. Three coded samples were served to the panelist. This method is useful in the quality control work to determine if different substitution or some other change in manufacturing results in a detectable difference in the product. Panelists were untrained but familiar with traditional pasta product. The analyses were performed in isolated booths under red light. The red light was used to mask dark color caused by addition of wheat germ. Macaroni were served with and without ketchup at the temperature at which they are normally consumed.

The cooked samples were served in coded dishes to panelists. Tables for analysis of triangle test data were obtained from Laboratory Methods for Sensory Evaluation of Food (Larmond, 1982).

In the triangle test each panelist found a degree of difference (slight, moderate, much and extreme) between the macaroni samples. Each difference has a constant value and tabulated in Table 2.1. In order to determine the average degree of difference between the samples, the number of panelists was multiplied by the corresponding difference value. Then calculated results obtained from each difference were summed together and divided into total number of judge that found the odd sample.

Table 2.1. Degree of difference and their values used in the triangle test

Degree of difference	Value
Slight	1
Moderate	2
Much	3
Extreme	4

b) Hedonic scale

Macaroni samples for sensory evaluation were cooked for 10 min, drained and served to the 12 panelists, who were untrained but familiar with traditional macaroni products. Panelists, using a hedonic scale of 1 to 7 judged the following parameters; appearance (1=pale, 4=normal, 7=too dark), texture (1=soft, 4=normal, 7=too chewy, firm), flavor (1=none, 4=normal, 7=too strong), over-all score (1=dislike, 7=like very much) (Buck, Walker & Watson, 1987). The analyses were performed in isolated booths.

2.2.5. Microbial analysis

2.2.5.1. Sample preparation

25 g macaroni samples were weighed aseptically in a sterile glass container. Then it was placed in 225 ml of a sterile peptone-water (0.1%) and mixed with magnetic stirrer to homogenize sample for 20 min (Jay, 1986).

2.2.5.2. Aerobic plate count (APC) and mold and yeast counts

Total bacteria and mold and yeast counts were made with the aerobic spread plate count method as described by Jay (1986). Aerobic plate count (PCA) for total bacteria and potato dextrose agar (PDA) for mold and yeast count was used for analysis.

A 0.2 ml of sample was transferred into corresponding labeled plate and spread plated over the agar surface. Inoculated PCA plates were incubated at 35 °C for 24 hr to 72 hr. Inoculated PDA plates were incubated at 25 °C for 2 to 5 days. Colonies on the plates in the range from 30 to 300 colonies were counted. Number of colony forming units were counted and average value was taken from duplicate counts. The number of CFUs were multiplied by a dilution factor and divided by inoculation amount to determine CFUs per gram of macaroni, then CFUs were transformed into corresponding logarithmic numbers.

2.2.6. Statistical analysis

SPSS 8.0 software for Window was used to perform statistical analyses. Differences in macaroni samples due to enrichment of raw and microwaved wheat germ were tested for significance using analysis of variance techniques (ANOVA). Duncan's multiple range test was used when the analysis of variance indicated significant difference in means. A level of significance of $P<0.05$ is used throughout the analysis.

CHAPTER III
RESULTS and DISCUSSION

3.1. Shelf life Studies of Whole Wheat Germ and Wheat Germ Oil

3.1.1. Accelerated shelf life test of whole wheat germ at 60, 70 and 80 °C

Although taste assessment is the most reliable method to evaluate the oxidative state of food lipids, it is an expensive and cumbersome procedure. Therefore, oil stability is usually determined by objective methods under accelerated conditions because ambient temperatures may demand excessively long period. Several accelerated methods of testing are generally used, among which the schaal oven test is the most frequently employed. Both chemical and instrumental techniques are employed to assess the oxidative state of the stored oil. These methods detect either the primary or secondary products of lipid oxidation and have been found to correlate well with descriptive sensory analyses (Abov-Ghorbia *et al.,* 2000). In this study the oxidative stability of whole wheat germ was studied by accelerated storage method of schaal oven test at 60, 70 and 80 °C. The development of peroxides was followed at specified intervals by measuring the PV up to the end of the storage time.

During storage the changes in PV and FFA was followed and results were shown in the Figures 3.1 and 3.2 respectively. There is no standard maximum limit of peroxide and free fatty acid value to determine shelf life of wheat germ. In literature, it was found a peroxide value of 20 meq KOH/kg of germ as threshold value, beyond which the rancidity was detected by smell and taste (Shurpaleker and Haridas Rao, 1977) and in this study, this value was used as an upper limit to end the shelf life of wheat germ.

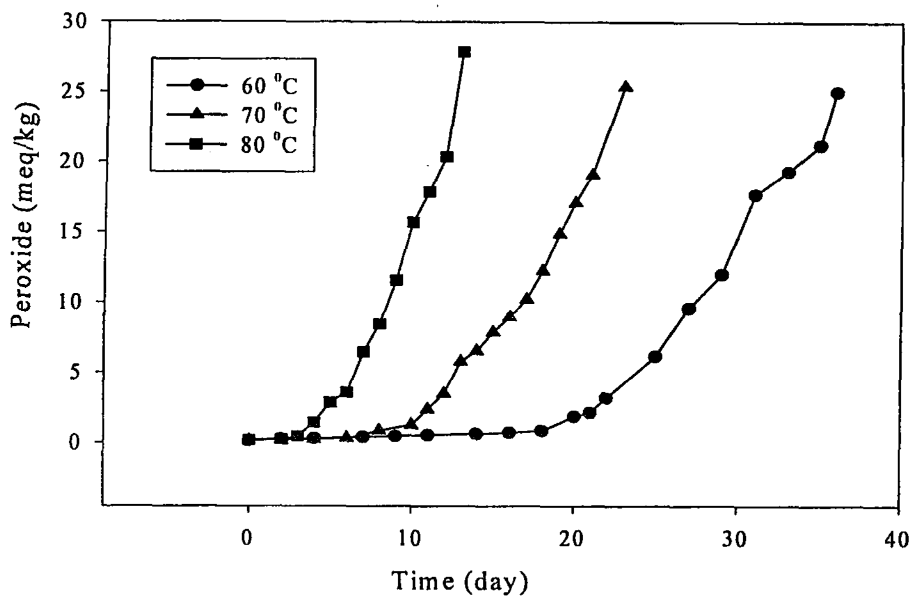

Figure 3.1. Changes in PV of whole wheat germ during storage at 60, 70 and 80 °C

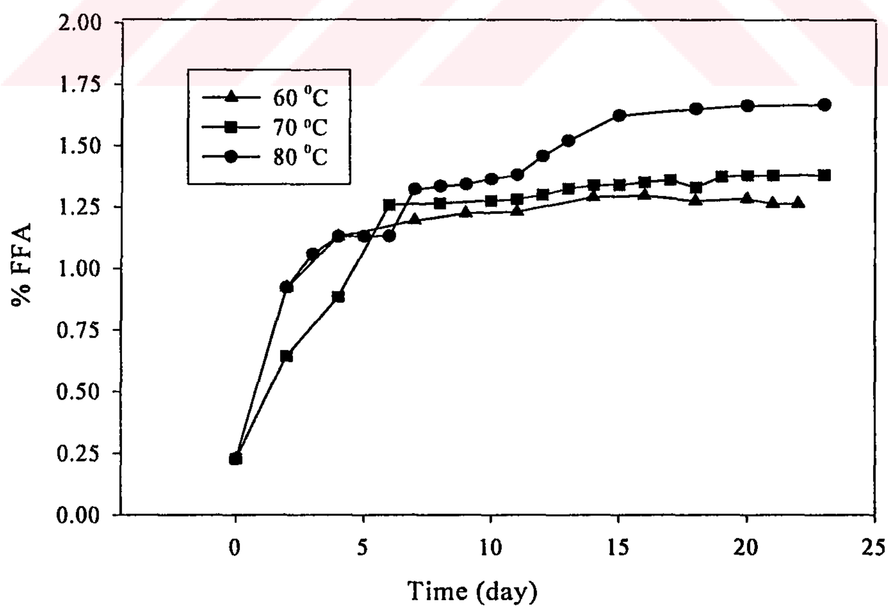

Figure 3.2. Changes in FFA of whole wheat germ during storage at 60, 70 and 80 °C

According to PV, shelf life of whole wheat germ obtained from storage at 60, 70 and 80 °C was 34, 22 and 12 day respectively and results were significantly ($P<0.05$) different from each other. As expected PV, value of wheat germ at 80 °C increased rapidly when compared PV values at the other temperatures.

In order to estimate the Arrhenius parameters of whole germ, two-step ordinary linear least squares fit was applied to experimental PV data at 60, 70 and 80 °C (Kaya, 2000). Assuming a first-order reaction for system during oxidation, the kinetic parameters can be calculated based on the model proposed in equation 3.1 and 3.2.

$$dC/dt = kC \qquad \textbf{(3.1)}$$

where C is the peroxide value (meq/kg) at time t, and k is the rate constant (day^{-1}).

$$k = k_0 \exp(-E_a/RT) \qquad \textbf{(3.2)}$$

where k_0 is the pre-exponential factor (day^{-1}), E_a is the activation energy (kJ/mol), T is the absolute temperature (K) and R is the gas constant (8.314 J/mol.K).

Rate constant (k) of each temperature was determined by plotting ln(C) versus time (equation 3.1 for a first order reaction) and results were shown at Figures 3.3, 3.4 and 3.5. The Arrhenius equation is often used as a theoretical basis for the development of a mathematical description of the temperature sensitivity of a product (equation 3.2).

E_a and k_0 was obtained by plotting ln(k) versus 1/T and represented at Figure 3.5.

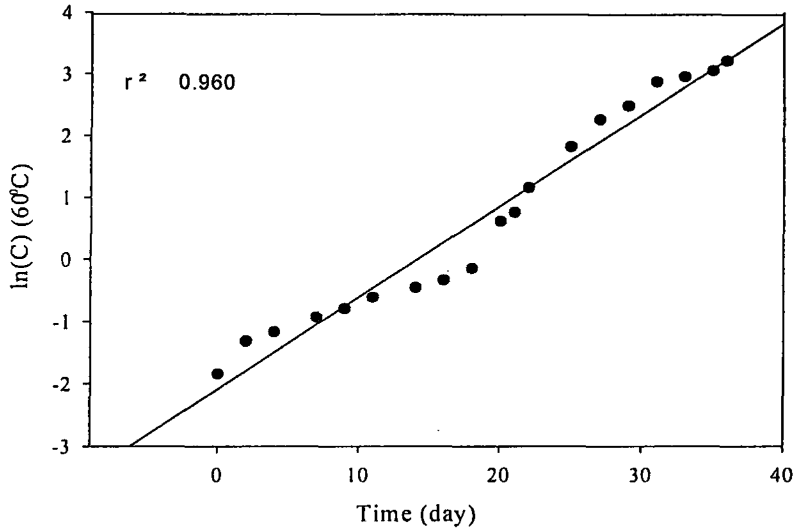

Figure 3.3. Plot of ln(C) versus time at 60 °C

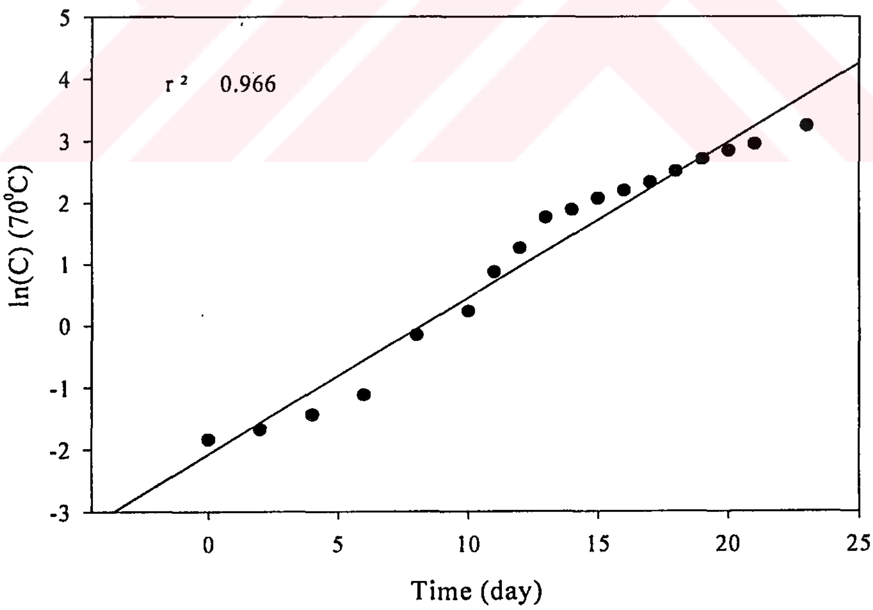

Figure 3.4. Plot of ln(C) versus time at 70 °C

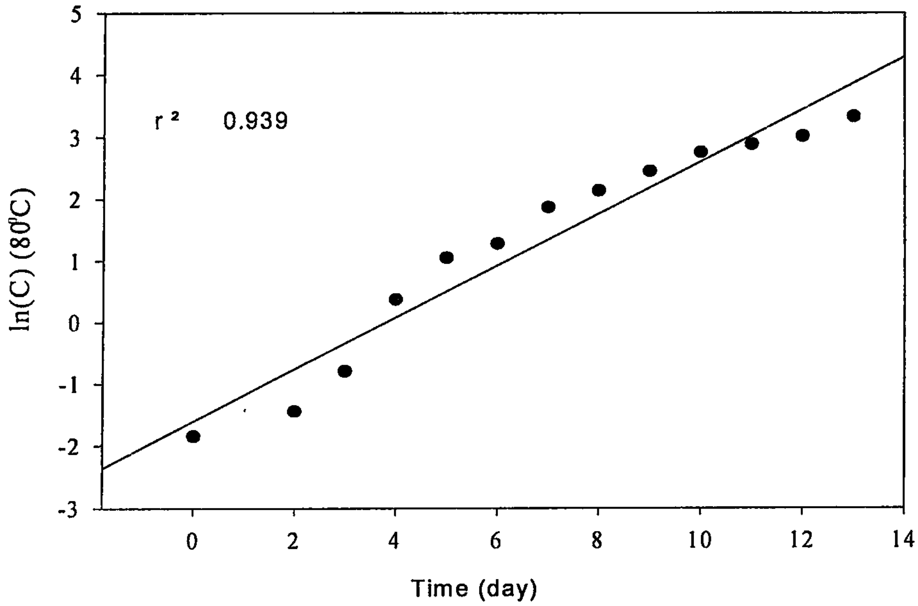

Figure 3.5. Plot of ln(C) versus time at 80 °C

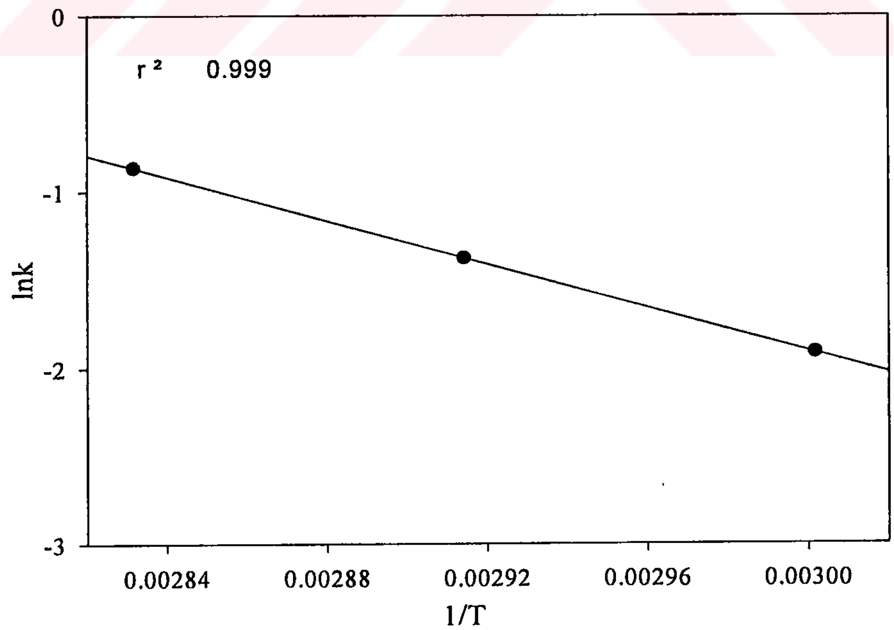

Figure 3.6. Plot of lnk versus 1/T

The Arrhenius parameters and the initial concentrations estimated for whole wheat germ using two-step linear least squares were tabulated in Table 3.1 for a first order kinetic.

Table 3.1. First order kinetic parameters of whole wheat germ from PV data

Parameters	60 °C	70 °C	80 °C
C_0	0.124 ±0.05	0.125±0.03	0.202±0.01
k(day^{-1})	0.148±0.01	0.253±0.02	0.421±0.01
R^2	0.960	0.967	0.939

E_a values of whole wheat germ was found as 51.13 kj/mol. The calculated value of E_a for whole wheat germ was lower than E_a of oils given in the literature. In the literature E_a of most oils varied in the range 63-89 kj/mol. E_a for corn, conola, soybean and sunflower oil was found as 77.78, 88.45, 79.66 and 63.81 (kJ/mol) respectively (Advaryu *et al.,* 2000). Low E_a of wheat germ may be explained by the storage of wheat germ as whole instead of its oil. Storage of whole wheat germ may result in a lower rate of oxidation than wheat germ oil due to lipid content and composition of wheat germ. The activation energy is observed to be influenced by the degree of poly-unsaturation present in the oil. A high poly-unsaturation (linoleic and linolenic acid content) would lower and high oleic content in the fatty acid chain would increase the E_a for oxidation (Advaryu *et al.,* 2000).

The rate of free fatty acid increase (FFA) during storage of wheat germ at 60, 70 and 80 °C was very low in contrast to the PV development. A slight increase was found in the FFA content of the samples as shown in Figure 3.2.

The FFA content of whole wheat germ samples increased from 0.23% to 1.26, 1.38 and 1.67% at the end of the storage at 60, 70 and 80 °C and statistically found significantly different ($P<0.05$) from each other. The FFA content reached a constant value of 1.13% for 60 °C after 4 day, 1.25% for 70 °C after 6 day and 1.32% for 80 °C after 7 day and after these times no significant increase was observed in FFA (Figure 3.2).

As expected, increase in FFA and PV were higher at 80 °C when compared to other two temperatures. The normal lipid oxidation pathway is very complex and is affected by temperature, oxygen and type of substrate (Labuza, 1971). The reason of increase in FFA and PV and formation of off-flavor during storage may be attributed to unsaturated fatty acid composition and also lipase and lipoxidase enzymes present in the wheat germ (Rao *et al.,* 1980).

3.1.2. Shelf life studies of whole wheat germ at 30 and 40 °C

Whole wheat germ samples were stored at 30 and 40 °C in order to determine the storage conditions of wheat germ at low temperatures and compare the results with results of accelerated shelf life test made at temperatures of 60, 70 and 80 °C. During storage, changes in PV and FFA were followed to determine effect of these temperatures (30 and 40 °C). Results were shown in the Figures 3.7 and 3.8 respectively.

PV of whole wheat germ stored at 30 and 40 °C reached to a maximum value of 20 meq/kg after 63 and 54 day respectively. At the end of this time FFA of whole wheat germ increased from 0.61 to 1.38% for 30 °C and 1.51% for 40 °C. As observed in the storage of whole wheat germ at 60, 70 and 80 °C, FFA displayed a constant value after 50 day for 30 °C and 30 day for 40 °C.

Results for storage time of whole wheat germ at 30 and 40 °C were compared with results of 60, 70 and 80 °C and shelf life values of whole wheat germ determined at 60, 70 and 80 °C was plotted against their storage temperatures (Figure 3.9). A linear line with an equation was obtained from this graph. The equation obtained from this line is given below

$$Y = 99.67 - 1.1\ X \qquad \textbf{(3.3)}$$

where Y is the time (day) and X is the temperature (°C).

By using equation 3.3, shelf life values of whole wheat germ for 30 and 40 °C were calculated. Calculated shelf life values of wheat germ obtained from equation (3.3) was 63 day for 30 °C and 54 day for 40 °C. These estimated values correlate well with the experimentally determined data. The experimentally detected shelf life of wheat germ obtained was 63 day for 30 °C and 54 day for 40 °C. It was concluded that, by using accelerated shelf life test we can determine shelf life of wheat germ rapidly at lower temperatures without making an experimental study.

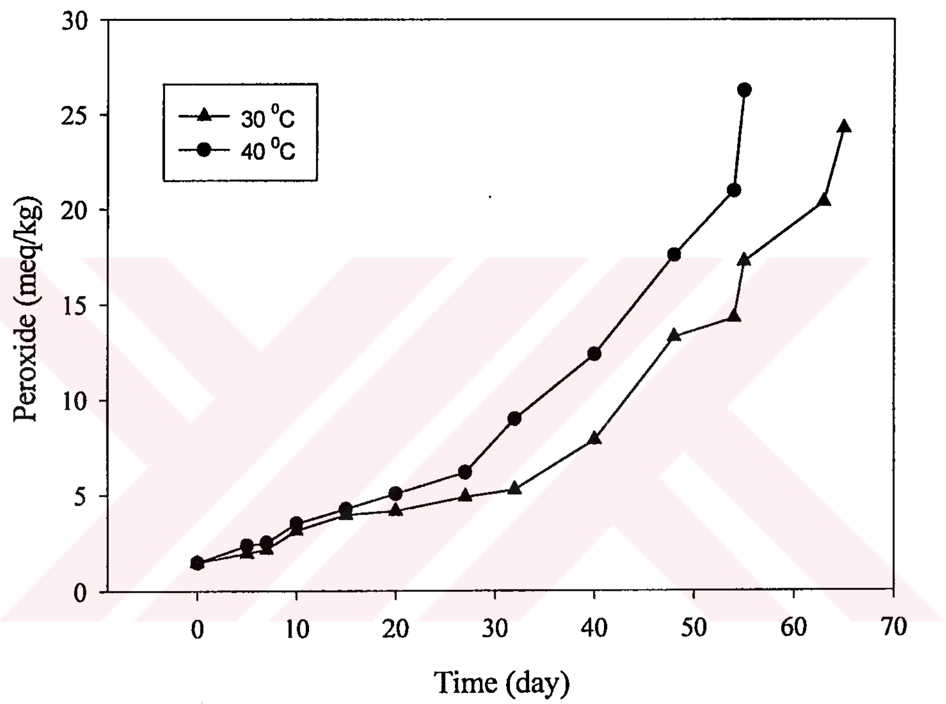

Figure 3.7. Changes in PV of whole wheat germ during storage at 30 and 40 °C

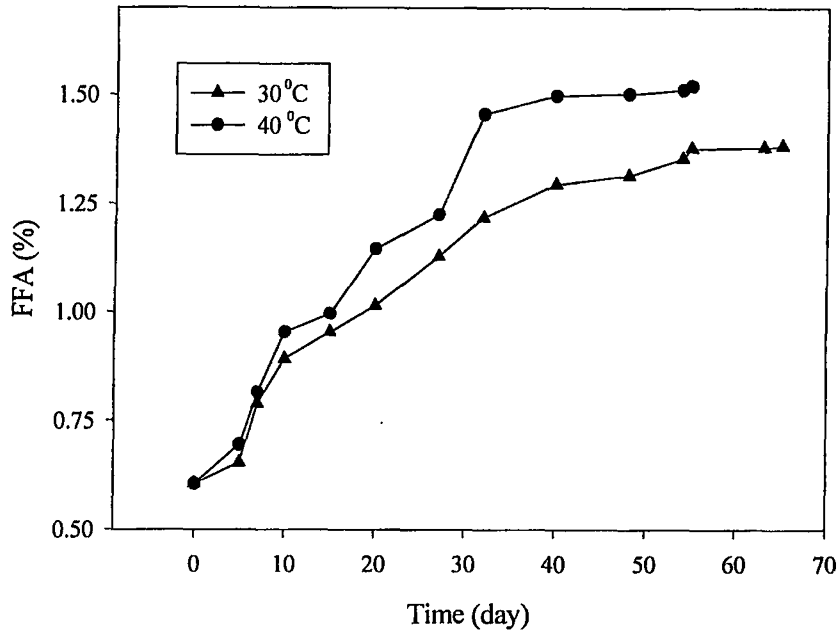

Figure 3.8. Changes in FFA of whole wheat germ during storage at 30 and 40 °C

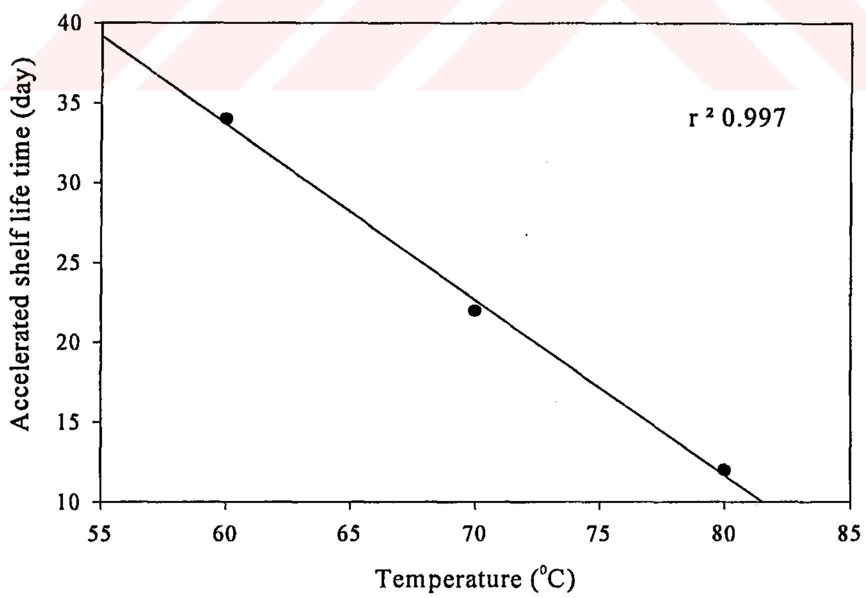

Figure 3.9. Plot of shelf life time of whole wheat germ versus temperature at 60, 70 and 80 °C

3.1.3. Shelf life studies of wheat germ oil at 30 and 40 °C

Figures 3.10, 3.11 and 3.12 show the change in the PV, FFA and TBA values of wheat germ oil during storage at 30 and 40 °C. There is no standard limit of PV for wheat germ oil as in the whole wheat germ. In this study PV of wheat germ oil was followed up to permitted limit of 10 meq/kg for most oils such as soybean and olive oil given by food codex (Anonymous, 2003).

PV of wheat germ oil reached its maximum value of 10 meq/kg at the end of 20 and 15 day at 30 and 40 °C, respectively. When these results were compared with whole wheat germ at the same temperatures it was found that shelf life of wheat germ oil was lower than shelf life of whole wheat germ that was 63 day at 30 °C and 54 day at 40 °C. The difference between the shelf life of whole wheat germ and wheat germ oil may be due to different PV for the end of shelf life. As explained above 20meq/kg was taken as standard limits for end of the whole wheat germ shelf life and 10 meq/kg was for wheat germ oil. If the 20 meq/kg would be taken as a basis for wheat germ oil, this value could be reached at the end of 40 day for 30 °C and 28 day for 40 °C (Figure 3.10). Still shelf life value of wheat germ oil was lower than whole wheat germ at both temperatures. This may be explained with the type and composition of the sample. Whole wheat germ contains 20.4% oil and unsaponifiable matter (USM) that consists of minor components accompanying triacylglycerols (sterols, tocopherols, lipopigments and hydrocarbons). It was found that USM retard oxidation in vegetable oils and model lipids subjected to heating. In whole material there are natural components that inhibit oxidation and they are removed to a great part from raw oil during extraction (Malecka, 2002). This may be reason to explain the difference between shelf life of whole wheat germ and wheat germ oil. In living tissues, labile acyl lipid constituents, such as unsaturated fatty acids, are sufficiently stable. Natural antioxidants and enzymes effectively prevent premature lipid oxidation. Once isolated from plant materials, lipids autoxidise readily. As a result, organoleptic and nutritional quality is reduced, and even toxic products may be formed. Autoxidation is a complex process, however model studies have revealed that the rate of autoxidation is affected by fatty acid composition, degree of unsaturation, the presence and activity of pro- and antioxidants, partial presence of oxygen, the surface exposed the oxygen (dispersed systems) and storage conditions (light, temperature, moisture content). Antioxidants inactivate reactive radicals at the initial steps of autoxidation, thus avoiding the propagation of the radical chain reaction (Krings and Berger, 2001; Krings *et al.*, 2000).

TBA values of wheat germ slightly increased during storage while the PV follows an exponential increase (Figure 3.12). Increase in TBA was higher at 40 °C than at 30 °C as expected. It increased from 0.38 to 0.7 mg malonaldehyde/kg at 30 °C and 1.5 malonaldehyde/kg at 40 °C at the end of the shelf life. A statistically significant ($P<0.05$) difference was found between TBA values of wheat germ stored at 30 and 40 °C. As can be seen in the Figure 3.13 a decrease was observed after an increase and it may be explained with the degradation of products such as malonaldehyde to the other high molecular weight compounds(Krings and Berger, 2001; Krings *et al.*, 2000).

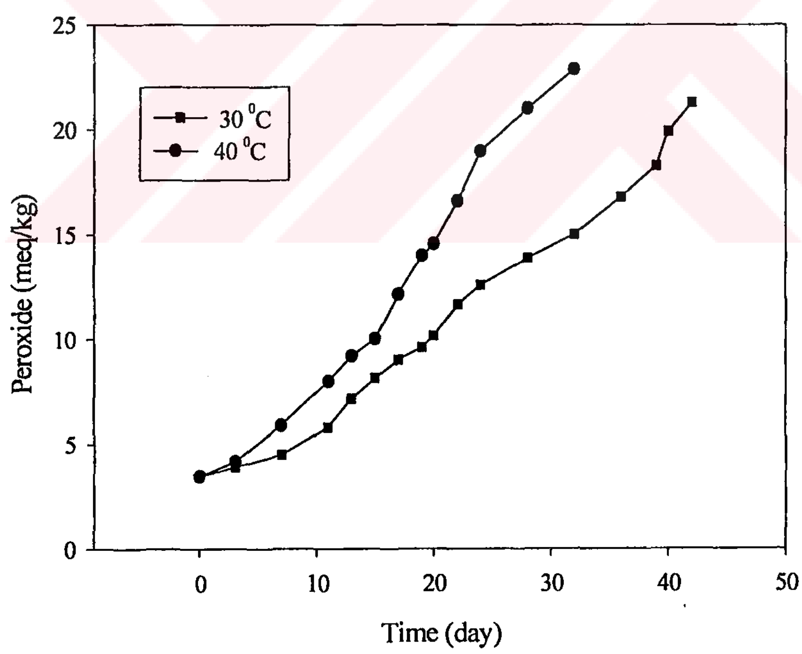

Figure 3.10. Changes in the PV of wheat germ oil during storage at 30 and 40 °C

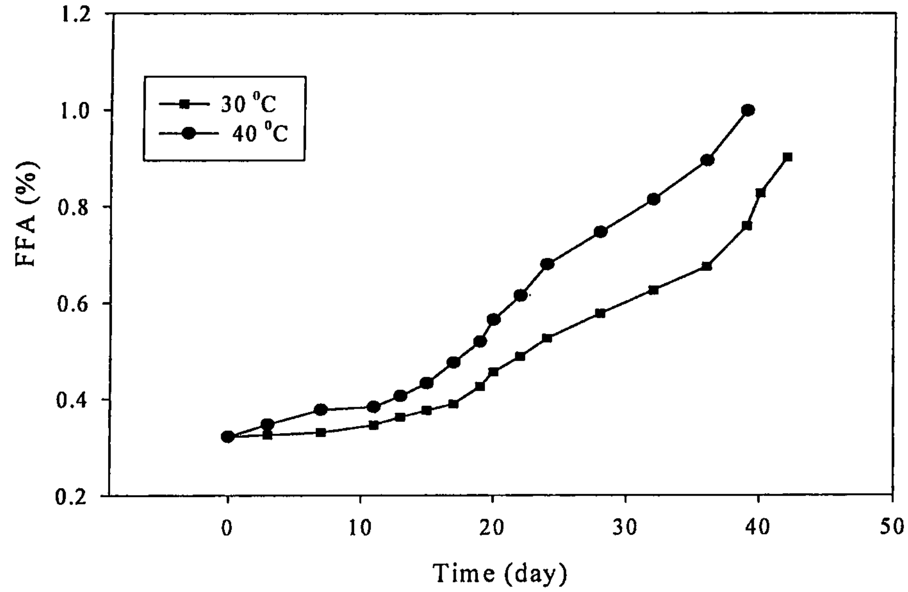

Figure 3.11. Changes in the FFA of wheat germ oil during storage at 30 and 40 °C

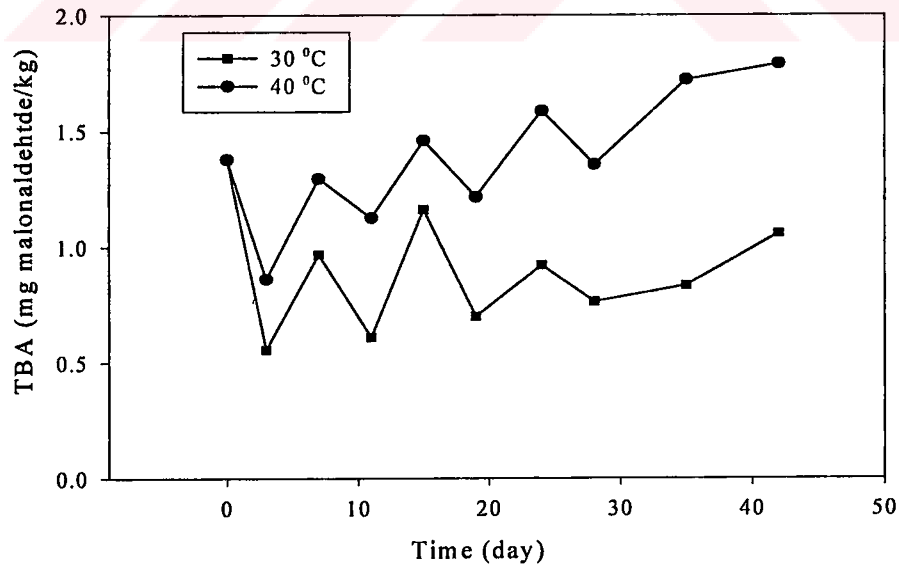

Figure 3.12. Changes in the TBA of wheat germ oil during storage at 30 and 40 °C

Malonaldehyde occurs in foods as an end product of oxidation of polyunsaturated fatty acids and its quantification is considered as an index of lipid peroxidation or oxidative rancidity. The malonaldehyde reacts with the TBA reagent to produce a red colored complex with a distinct absorption maxima at 532 nm (Setiowaty. and Man, 2003).

Although other degradation mechanisms are also possible, the oil degradation process has been generally established as being a free radical mechanism yielding hyroperoxides, also called primary oxidation products, which in turn degrade into aldehhydes, and ketones, lactones, alcohols, acids, etc., or secondary oxidation products. The methods used to determine the rate at which oxidation process are related to the measurement of the concentration of primary or of secondary oxidation products or of both or to the amount of oxygen consumed during the process. Among those based on the evaluation of the concentration of primary oxidation products, peroxide value (PV), which measures hyroperoxide concentration, is one of the most widely used (Gullién and Cabo, 2002). The peroxides are intermediate products in the formation of carbonyl and hyroxy compounds and very sensitive to temperature changes (Gray, 1978). Some of the other methods are based on the concentration of secondary oxidation products including aldehydes, ketones, acids, alcohols, lactones, ethers, hydrocarbon and furan derivatives. Determination of malonaldehyde content is the basis of the so called thiobarbituric acid test (or TBA) is another method. These methods measure the oxidative stability with different degrees of approximation and gives information on the concentration of the corresponding compound or group of compounds at different moments during oxidation process (Gullién and Cabo, 2002).

FFA % of wheat germ oil increased from 0.32 to 0.46 after 20 day storage at 30 °C and to 0.43 after 15 day storage at 40 °C. As can be seen, the increase in FFA in wheat germ oil was approximately similar and lower than the FFA content of wheat germ stored at same temperatures. This may be due to effect of high amount of moisture present in the wheat germ when compared to its oil. Moisture is one of the important parameter for hydrolysis of fats into FFA.

Finally, induction period (IP) of the raw wheat germ and wheat germ oil was found by using their plot of PV at each temperature and tabulated in Table 3.2. It was found that shelf life values of the samples reached to 10 meq/kg PV rapidly after their induction period. IP of raw wheat germ stored at 60 °C was 20 (day) and at this time PV was 1.85 meq/kg then showed rapid increase and reached to 10 meq/kg and 20 meq/kg after 8 and 14 day respectively (Table 3.2).

Table 3.2. Induction period and shelf life values of raw wheat germ and raw wheat germ oil

Type of Sample	Storage Temperature (°C)	Induction Period (day)	Peroxide Value* (meq/kg)	Shelf life at 10 meq/kg (day)	Shelf life at 20 meq/kg (day)
Raw Wheat Germ	60	20	1.85	28	34
	70	11	2.38	17	22
	80	5	2.85	9	12
	30	38	6.12	45	63
	40	28	6.65	35	54
Raw Wheat Germ oil	30	14	7.85	20	40
	40	12	8.25	15	28

*Peroxide value corresponding to their induction period.

Wheat germ which shelf life properties were determined was used for enrichment of macaroni.

3.2. Determination of Some Chemical, Physical and Sensory Properties of Macaroni Enriched with Raw and Microwaved Wheat Germ.

Our previous work shown in Table 3.3 (İbanoğlu, Pınarlı, Dalgıç & Öner, 2000) revealed that beyond 20% wheat germ addition, macaroni samples had high cooking losses and ash content, which is not suitable for macaroni standards (Anonymous, 1989). However at 15% or less wheat germ supplementation, cooking loss and ash content were in the range of Turkish Specifications (Anonymous, 1989). Therefore, in this study 15% wheat germ was used for enrichment of macaroni.

Table 3.3. Effect of wheat germ addition on composition of macaroni and standard values for macaroni

Amount of added wheat germ (%)	Moisture (%)	Ash (%)	Protein (%)
0	10.3	0.76	10.5
5	10.5	0.87	11.4
10	10.1	1.02	12.3
15	10.8	1.26	13.0
20	10.2	1.45	13.8
TSE value for Macaroni	13^b	1^b	10.5^a
TSE value for Fortified macaroni	13^b	1.5^b	11^a

a : minimum value
b : maximum value.

3.2.1. Chemical composition

Results showed that composition of macaroni was effected by wheat germ addition. A significant ($P<0.05$) difference was obtained between control and enriched type of macaroni in the composition (Table 3.4). Addition of raw wheat germ (RG) and microwaved wheat germ (MG) significantly increased the protein content of control macaroni (CM) from 11.4 to 13.3%. Enrichment of durum semolina with RG and MG containing 3.8% ash content resulted in an increase in macaroni ash values up to 1.5%. Upon the addition of RG and MG, the crude oil content of CM increased from 2.5 to 5.2%, which may affect the shelf life of macaroni for a long storage period. It was reported that wheat germ contains 84% unsaturated fatty acids. Approximately half of the fatty acid was linoleic acid (53%). Also wheat germ contains 3.6% linolenic acid and 28.1% oleic acid (Shurpaleker & Haridas Rao, 1977). Macaroni samples were analysed during one-year storage.

Table 3.4. Composition of raw material and macaroni samples (%)[A]

Type	Moisture	Protein (Nx5.7)	Crude Oil	Ash	Carbohydrate (by difference)
Semolina	10.0± 0.007 (c)	11.1± 0.141 (c)	2.4± 0.014 (c)	0.9± 0.07 (c)	75.6 ± 0.058 (a)
Wheat germ	11.5± 0.005 (a)	26.6± 0.071 (a)	20.4± 0.052 (a)	3.9± 0.07 (a)	39.9± 0.049 (c)
CM	11.4± 0.071 (a)	11.5± 0.072 (c)	2.3± 0.06 (c)	0.9± 0.08 (c)	73.6± 0.070 (a)
RM	10.5± 0.012 (b)	13.4± 0.071 (b)	5.2± 0.071 (b)	1.5± 0.06 (b)	69.5± 0.053 (b)
MM	10.5± 0.013 (b)	13.4± 0.140 (b)	5.2± 0.052 (b)	1.5± 0.06 (b)	69.5± 0.066 (b)

[A]Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.
CM = Control macaroni, produced with 100% durum semolina; RM = Macaroni produced with 15% raw wheat germ and 85% durum semolina;
MM = Macaroni produced with 15% microwaved wheat germ and 85% durum semolina.

3.2.2. Color

Color of pasta products is an important indicator of quality. Consumers tend to be attracted by products of amber-yellow color (Kobrehel *et al.*, 1974). The high yellow pigment content of durum semolina is one of the desirable attribute in the production of macaroni. Since the desirable color for the processed product is clear bright yellow, factors which discolor it are undesirable (Matsuo and Irvine, 1967).

Color measurement results were summarized in the Table 3.5. It was found that color was significantly ($P<0.05$) affected with addition of wheat germ. To illustrate the effects on appearance caused by addition of RG and MG, pictures of the products are shown in Figure 3.13. As can be seen in this figure, enriched macaroni samples showed a significant decrease in color scores compared to the control sample. Kobrehel et al., (1974) found that improvement of cooking quality with the increase of protein content could cause the color deterioration of pasta products.

Figure 3. 13. Macaroni made with 100% semolina, 15% raw or microvawed wheat germ

Results showed that there is an increase in the darkness of enriched macaroni samples. With the addition of raw and microwaved wheat germ the brightness index (L value) of control decreased from 41.4 to 33.6 and 30.3, respectively. There are some factors that influence the appearance of the macaroni other than its size and shape are its color, uniformity and surface texture (Oliver *et al.*, 1993). It is well known that yellow and brown indices of macaroni can arise due to a Maillard type reaction, an enzymatic reaction or increase in ash content, while the red index is strictly related to the Maillard reaction (Matsuo and Irvine, 1967; Acquistucci, 2000). Wheat germ was rich in essential amino acids lysine, methionine and threonine. Sucrose is a reducing sugar with 80-90% of total sugars in wheat germ. It also contains peroxidase and lipoxidase enzyme (Shurpaleker & Haridas Rao, 1977). It was reported that natural pigments (xanthophyll, taraxantin, and flavin) of amber durum wheat was oxidized by lipoxyganeses (Fernandes, *et al.*, 1979). Darkening in the color of enriched macaroni samples may be due to Maillard reaction or activity of enzymes present in wheat germ. Nonenzymatic browning related to the Maillard reaction occurs easily during drying. The protein-carbohydrate reactions, which involve the terminal amino group of free amino acids, proteins and reducing sugars, lead to change in the native structure of protein and degradation of amino acids.

Among the essential amino acids, lysine was shown to be involved to a greater extent in these type reactions with respect to the other amino acids (Acquistucci, 2000).

Table 3.5. Hunter color measurement of macaroni samples[A]

Type	L	a	b
CM	41.4±0.007 (a)	4.6±0.007 (c)	18.4±0.007 (a)
RM	33.6±0.014 (b)	6.2±0.070 (b)	14.9±0.007 (b)
MM	30.3±0.007 (c)	7.2±0.007 (a)	12.4±0.007 (c)

[A]Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

There is an increase in the redness of enriched macaroni samples when compared to control. As mentioned earlier, the red component is the result of non enzymatic browning related to Maillard or carbonyl-amino, reactions developed between proteins and sugars (Feillet, Autran & Icard-Verniére, 2000; Acquistucci, 2000; Acquistucci, 1996; Matsuo & Irvine, 1967). The redness (a value) of control was increased from 4.6 to 6.2 for RM and 7.2 for MM. With the addition of raw and microwaved wheat germ, yellowness (b value) of control decreased from 18.4 to 14.9 and 12.4 respectively. As can be seen from the results (Table 3.3), brightness and yellowness were lower and redness was higher for MM (macaroni produced with 15% microwaved wheat germ) than for CM and RM (macaroni produced with 15% raw wheat germ), due to possibly additional heating of RG in the microwave oven before macaroni production. The yellowness of pasta is affected by different factors; which are intrinsic to the quality of semolina (natural carotenoid pigments, protein, ash and lipoxiganase activity) and processing conditions (Debbouz, 1994; Borelli *et al.*, 1999). The brown color tends to mask the yellow color when it reaches substantial values (Feillet *et al.*, 2000).

Kordonowy and Youngs (1985) reported that the addition of durum bran to semolina created spaghetti darker than that of the semolina spaghetti. In a similar study of Bahnassey and Khan (1986), it was found that fortified spaghetti with legume flour or protein concentrate decreased the color scores. The largest decrease in color scores was observed for roasted and nonroasted navy bean protein concentrates. Likewise Bergman, Gualberto and Weber (1994) reported that supplementation of durum semolina with cowpea resulted in an increase in darkness, yellowness, and redness as well as decrease in greenness.

3.2.3. Sensory evaluation

3.2.3.1. Hedonic scale

Results of sensory evaluation are given in Table 3.6. It appears that raw and microwaved wheat germ addition significantly ($P<0.05$) affected the flavor of macaroni. Since MM has the highest flavor score when compared to the CM and RM, RM were preferred more than MM in terms of flavor. This may be due to slightly roasted flavor of MM, which was caused by microwave heat treatment of wheat germ.

Similar trends were observed within appearance scores. Enriched macaroni samples were scored higher for their appearance when compared to control macaroni. Higher score of RM and especially of MM indicates dark color and it was due to enzymatic and non enzymatic reactions with high amount of carbohydrate, proteins and enzymes present in wheat germ as explained before. Darker color of the MM compared to RM was possibly due to effect of microwave treatment of wheat germ.

Pearson correlation test was carried out to determine the correlation between appearance scores and L values (brightness index) of Hunter color measurement. Results showed that there is significant ($P<0.05$) negative correlation ($r=-0.985$) between appearance sensory score and L values of macaroni samples. It means as brightness index (L) decreased, appearance score given by panelists increased and indicates darkening of the color of macaroni.

There was no significant ($P<0.05$) difference between the means of the texture and overall score of macaroni samples. The same preference was observed by the panelists for textural and overall attributes of macaroni samples. Although panelists gave the higher flavor scores for RM and MM when compared with CM, it was surprising that there was no significant difference between overall scores of samples. Results revealed the fact that, consumers may prefer RM and MM although some significant differences in flavor and appearance were observed.

Table 3.6. Sensory evaluation of macaroni samples after two different storage periods[A]

	Flavor	Appearance	Texture	Overall score
Initial				
CM	3.5±0.7 (c)	2.4± 1.2 (c)	3.7±0.5 (a)	4.8±1.1 (a)
RM	4.4±0.5 (b)	2.4± 1.2 (c)	4.3 ±0.7 (a)	4.7±0.8 (a)
MM	5.5±0.8 (a)	6.4±0.6 (a)	4.3 ±1.3 (a)	4.4 ±1.6 (a)
12 months				
CM	3.4±0.7 (c)	2.9±1.1 (c)	3.8±1.0 (a)	4.8±1.9 (a)
RM	4.3±0.4 (b)	4:9±0.8 (b)	3.8±0.9 (a)	4.5±1.7 (a)
MM	5.5±0.6 (a)	6.2±0.6 (a)	3.7±1.4 (a)	4.2±0.9 (a)

[A]Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

Results of sensory evaluation after one-year storage were similar with results of initial sensory evaluation (Table 3.6). It was found that storage of enriched macaroni samples up to one year did not affect significantly ($P<0.05$) the result of taste panel evaluation.

3.2.3.2. Triangle test

Three coded samples were given to the panelists. They were told that two of the samples are the same and one was different and asked to identify the odd sample. Triangle test was used to determine if ingredient substitution resulted in detectable difference in the product.

Analysis of the results of triangle test was based on the probability that if there were no detectable difference, the odd sample would be selected by chance one-third of the time.

3.2.3.2.1. Comparison of control macaroni with macaroni produced with 15% raw wheat germ

a) Macaroni samples served without ketchup

Triangle test results showed that there was slight difference between the macaroni produced from 15% raw wheat germ and control. 15 of the 22 panelists who correctly identified the odd sample, 7 found the control sample more acceptable, 4 found the macaroni produced from 15% raw wheat germ more acceptable and 4 found the both type have the same acceptability. According to statistical chart (Larmond, 1982), for a two-sample test (there are only two choices at this point), 7 was below the number required for significance at the 5% level. So, there were no macaroni that was significantly more acceptable over the other.

According to results, macaroni produced from 15% raw wheat germ has the same acceptability as control macaroni. Differences in the taste were calculated as described in section 2.2.3.12 and found as slight (Table 3.7).

Table 3.7. Degree of differences for each type of macaroni

	Panelists' preference (who correctly identify odd sample)				
Sample	Slight (1)*	Moderate (2)*	Extreme (3)*	Much (4)*	Degree of difference calculated
RM [a]	10	5	0	0	(10*1+5*2)/15=1.3 (slight)
RM with ketchup[a]	8	2	0	0	1.0 (slight)
MM [a]	6	13	2	0	1.8 (moderate)
MM with ketchup[a]	6	4	0	0	1.4 (slight)
RM [b]	7	3	0	0	1.3 (slight)
RM with ketchup [b]	9	1	0	0	1.1 (slight)
MM [b]	4	5	0	0	1.6 (moderate)
MM with ketchup [b]	5	2	0	0	1.3 (slight)

[a]; Initial
[b]; After one year storage
*; Correspondence score
See Table 3.4 for abbreviations.

b) Macaroni samples served with addition of ketchup

10 correct judgments out of 12 in a triangle test indicated that there was a significant difference at 5% level determined from statistical chart (Larmond, 1982).

4 of the 10 panelist who correctly determined the odd sample found the control more acceptable, 3 found the macaroni produced from 15% raw wheat germ more acceptable and 3 found the both samples have the same acceptability. According to statistical chart (Larmond, 1982), for a two-sample test, 4 was below the number required for significance at 5% level. This result showed that, macaroni produced from 15% raw wheat germ, which served with ketchup, has the same acceptability with the control. The degree of difference was slight as in the direct serving of macaroni (Table 3.7).

3.2.3.2.2. Comparison of control macaroni with macaroni produced with 15% microwaved wheat germ.

a) Macaroni samples served without ketchup

Moderate difference (Table 3.7) was found between the macaroni produced with 15% microwaved wheat germ and control.

Panelists were not correctly identified the more acceptable type of macaroni. According to statistical chart, number of agreeing judgments necessary to establish significant differentiation is lower than given number, so there were no macaroni which is significantly more acceptable over others. Moderate difference in the taste did not affect the acceptability of macaroni produced with 15% microwaved wheat germ. Both types were accepted at the same degree.

b) Macaroni samples served with ketchup

10 of 12 panelists, who correctly identified the odd sample in a triangle test, indicated that there was a significant difference at 1% level determined from statistical chart (Larmond, 1982). Slight difference (Table 3.7) was found between these two types of macaroni.

4 panelists found the control samples more acceptable, 4 found the macaroni produced with 15% microwaved wheat germ more acceptable and 2 found the both samples have the same acceptability. According to statistical chart (Larmond, 1982), for a two-sample test, 4 are below the number required for significance at 5% level. This result showed that, macaroni produced with 15% microwaved wheat germ, which served with ketchup, has the same acceptability with the control.

Some of the panelist preferred enriched type of macaroni much more than control. Addition of ketchup didn't musk the flavor of enriched macaroni. Although the number of panelist who determined correctly the odd sample decreased by serving macaroni with ketchup, still slight difference was found between enriched and control macaroni samples.

When these initial results were compared with the results of triangle test that was made after one-year storage, it was found that the degree of difference between the control and enriched type of macaroni did not change significantly. Storage up to one year did not significantly change the sensory of enriched macaroni.

The results of triangle test were similar with the results of hedonic scale. As a result enriched macaroni samples with raw and microwaved wheat germ were equally preferred with control macaroni.

3.2.4. Cooking quality

The quality of cooked spaghetti depends upon several factors such as amount of water absorbed, the loss of solids to the cooking water, and in particular, the firmness (Walsh, 1971; Olfat *et al.*, 1993). Dexter and Matsuo (1979) reported that to gain meaningful assessment of cooking spaghetti properties, measurements should be made at more than one cooking time. In our work, the characteristics of macaroni cooked for 10, 20 and 30 min are studied during one-year storage.

3.2.4.1. Weight increase

Weights of cooked macaroni samples were measured at initial, after six and twelve months of storage and weight increase was calculated according to the equation (2.1). ANOVA test showed that weight increase was significantly ($P<0.05$) affected by cooking time and wheat germ addition. Results suggest that water absorption of the macaroni was altered with addition of raw and microwaved wheat germ. Duncan's multiple range test was applied to compare means of the results (Table 3.8). As the cooking time increased, higher the weight increase was obtained. Storing the macaroni samples up to one year did not significantly ($P<0.05$) affected the weight increase of macaroni samples.

Table 3.8. Weight increase (%) of cooked macaroni samples at different cooking times and after different storage periods [A]

Storage Time	Type	Cooking Time (min) 10	20	30
Initial	CM	180.2±0.62 (a, C)	235.4±0.11 (a, B)	292.4±0.12 (a, A)
	RM	174.2±0.01 (b, C)	224.3±0.54 (b, B)	283.5±0.09 (b, A)
	MM	154.8±0.69 (c, C)	209.9±0.54 (c, B)	271.0±1.32 (c, A)
6 months	CM	181.4±0.14 (a, C)	237.4±0.18 (a, B)	291.8±0.49 (a, A)
	RM	176.2±1.13 (b, C)	226.0 ±1.03 (b, B)	286.2 ±0.73 (b, A)
	MM	154.8±1.03 (c, C)	211.9±0.85 (c, B)	272.1±0.94 (c, A)
12 months	CM	181.7±0.62 (a, C)	236.3±1.41 (a, B)	292.8±0.51 (a, A)
	RM	177.2±1.38 (b, C)	226.3±1.38 (b, B)	284.5±0.37 (b, A)
	MM	155.3±0.11 (c, C)	211.9±0.98 (c, B)	271.1±0.47 (c, A)

[A]Figures in the same raw sharing a common capital letter in parenthesis are not significantly different at 0.05 level
Figures in the same column sharing a common a letter in parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

The percent increase in weight of cooked macaroni decreased with RG and MG enrichment. The results are in agreement with the studies of Paulsen (1961), Kordanawy and Youngs (1985), Buck *et al.*, (1987), Duszkiewicz-Reinhard *et al.*, (1988), Sharma *et al.*, (2002) for fortified type of macaroni.

Weight increase of control macaroni was 180.2% after 10 min cooking and reached to 292.4% at the end of the 30 min. Macaroni enriched with raw wheat germ had a 174.2% weight increase and macaroni with microwaved wheat germ had a 154.8% weight increase after 10 min cooking and their weight increase reached to 283.5 and 271% at the end of 30 min cooking respectively. As can be seen from the results, weight increase was low in enriched macaroni samples especially in macaroni produced with microwaved wheat germ.

The reason for decrease in weight increase can be explained by the decrease in starch content (Lucisano, *et al.*, 1984) with addition of wheat germ and effect of heat on wheat protein structure during drying of macaroni samples. Heat is known to cause protein molecules to unfold, exposing functional groups previously within the molecule to its external environment. Such an occurrence may increase protein surface hyrophobicity, thus it decreases its water binding capacity (Bergman *et al.*, 1994). Effect of heating on water binding capacity of wheat germ can obviously be seen in MM. Weight increase in MM was significantly ($P<0.05$) lower than CM and RM due to possible denaturation of wheat germ proteins during microwave treatment and resulted in less water gain. Similar results were reported by Bergman *et al.*, (1994), Bahnassey and Khan (1986), and Neilsen *et al.*, (1980).

Bergman *et al.*, (1994) found that as with most ingredients used in food processing, a level exists at which the ingredient added provides an optimum effect. However, at the some point less than or greater than the optimum quantity, either the desired change in functionality does not occur or an unacceptable change results. They found that the addition of cowpea meal and soft wheat flour in to semolina for macaroni production a level is important. At a certain level, somewhere between 20 and 30% an effect on cooked weight occurred; at levels below this, they found no effect.

3.2.4.2. Volume increase

ANOVA showed that volume increase (swelling) of macaroni was significantly ($P<0.05$) affected by cooking time and addition of wheat germ. It was found that storage period had no significant affect ($P<0.05$) on swelling (Table 3.9) of macaroni samples. Results indicated that the weight and volume of enriched macaroni increased to at least twice the original weight and volume.

In this study a decrease in volume was found after a certain cooking period. Volume of macaroni increased at 10 min cooking and the increase in the volume continued up to 20 min. Since the samples were very soft and they lost their original swollen shape, a decrease in swelling at 30 min cooking was observed.

Volume of control macaroni increased to 76.6% at the end of 20 min cooking and showed a decrease to the 57.9% after 30 min cooking. Volume of RM reached to the 94.3% at the end 20 min cooking and showed a decrease to the 75.2% after 30 min cooking. MM showed an 86.5% increase in volume after 20 min cooking and its volume decreased to 65.3% at the end of 30 min cooking.

As can be seen from the Table 3.9 enriched macaroni samples had a higher volume increase than control macaroni and maximum volume increase was found in RM. This may be explained by the cellulose content (10-12%) of wheat germ. It increases the water absorption and so resulted in higher volume increase compared to CM. High water absorption is a desirable property in macaroni (İbanoğlu *et al.,* 2000).

Monad *et al.*, (1980) found that macaroni supplemented with 4% and 6% lupin flour showed a higher volume increase than the control. Also Olfat *et al.*, (1993) reported similar results concerning the effect of enrichment with a cellulose-derivative protein complex on macaroni. They found that enriched macaroni samples showed higher volume increase than control macaroni and also after 30 min cooking both control and enriched macaroni showed a decrease in percent swelling as observed in this study.

Table 3.9. Volume increase (%) of cooked macaroni samples at different cooking times and after different storage periods [A]

Storage Time	Type	Cooking Time (min) 10	20	30
Initial	CM	55.7±0.32 (c, C)	76.6±0.43 (c, A)	57.9±1.11 (c, B)
	RM	87.8±0.74 (a, B)	94.3±0.41 (a, A)	75.2±1.44 (a, C)
	MM	70.9±1.05 (b, B)	86.6±0.60 (b, A)	65.3±1.19 (b, C)
6 months	CM	55.7±0.81 (c, C)	76.1±1.51 (c, A)	57.9±1.04 (c, B)
	RM	85.5±1.42 (a, B)	96.3±1.41 (a, A)	76.6±0.95 (a, C)
	MM	70.7±0.64 (b, B)	86.7±2.14 (b, A)	63.8±0.57 (b, C)
12 months	CM	53.7±0.75 (c, C)	77.3±0.23 (c, A)	57.5±0.60 (c, B)
	RM	86.1±1.41 (a, B)	95.5±1.38 (a, A)	75.3±0.17 (a, C)
	MM	69.4±0.60 (b, B)	87.1±0.12 (b, A)	62.9±1.28 (b, C)

[A]Figures in the same raw sharing a common capital letter in parenthesis are not significantly different at 0.05 level
Figures in the same column sharing a common a letter in parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

3.2.4.3. Cooking loss

Cooking time and wheat germ addition affected significantly (P<0.05) on cooking loss of macaroni samples (Table 3.10). Storing macaroni samples up to one year did not significantly (P<0.05) affect cooking loss.

Enrichment of macaroni with RG and MG was resulted in higher cooking loss then CM. There is no significant (P<0.05) difference between cooking loss of RM and MM. Although microwaving the wheat germ significantly affected the weight and volume increase of macaroni sample, microwave cooking of wheat germ did not significantly affect the cooking loss.

Table 3.10. Cooking loss (%) of cooked macaroni samples at different cooking times and after different storage periods [A]

Storage		Cooking Time (min)		
Time	Type	10	20	30
Initial	CM	5.7±0.06 (b, C)	07.8±0.01 (b, B)	10.7±0.01 (b, A)
	RM	8.7±0.56 (a, C)	11.6±0.18 (a, B)	13.8±0.39 (a, A)
	MM	8.5±0.17 (a, C)	11.7±0.24 (a, B)	14.1±0.12 (a, A)
6 months	CM	5.4±0.21 (b, C)	07.7±0.38 (b, B)	11.1±0.18 (b, A)
	RM	8.7±0.31 (a, C)	11.3±0.11 (a, B)	14.0±0.25 (a, A)
	MM	8.3±0.18 (a, C)	11.3±0.32 (a, B)	13.8±0.47 (a, A)
12 months	CM	5.3±0.18 (b, C)	8.4±0.021 (b, B)	11.0±1.05 (b, A)
	RM	8.5±0.38 (a, C)	10.9±0.49 (a, B)	13.7±0.43 (a, A)
	MM	8.5±0.13 (a, C)	10.9±0.39 (a, B)	13.5±0.50 (a, A)

[A]Figures in the same raw sharing a common capital letter in parenthesis are not significantly different at 0.05 level
Figures in the same column sharing a common a letter in parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

Cooking loss was 5.7%, 8.7% and 8.5% for control, RM and MM for 10 min cooking respectively and reached 10.7%, 13.8% and 14.1% for control, RM and MM. It was demonstrated that, determination of residue in cooking water does not correlate well with spaghetti quality (Dexter and Matsuo, 1979). But in some other studies it was indicated that together with water absorption and swelling, cooking loss was the main criteria used for assessing the quality of cooked pasta (Lai, 2001, Sharma *et al.*, 2002). Cooking loss was related to the breakdown of pasta during cooking and has been considered as indicator of overall pasta cooking performance (Sharma *et al.*, 2002).

Sharma *et al.*, 2002 found that there was a significant reduction in cooking loss in the partially waxy lines, which is attributed to their lower amylose content because amylose is main constituent of the starch that leaches into the cooking water. They found a highly positive association between cooking loss and content of starch.

In all cases increase in cooking time was accompanied with increase in cooking loss. Effect of time on cooking loss can be explained by the thermal effect of excessive heating on the protein matrix. Modification in protein solubility may arise from changes in degree of association between polypeptide chains, hydrogen bonds, hydrophobic interactions, salt-linkage or disulfide covalent bonds (Feillet, Fevre & Kobrehel, 1974). These results are consistent with the results reported by Molina *et al.*, (1982), Kordonowy and Youngs (1985), Bahnassey and Khan (1986), Olfat *et al.*, (1993), Wang *et al.*, (1999) for fortified type of macaroni.

Increase in the cooking loss due to enrichment of macaroni with RG and MG may be related to gluten dilution and the water soluble protein fraction of wheat germ. It was demonstrated that a linear relationship exists between spaghetti cooking quality and protein content (Dexter & Matsuo, 1979) and increasing amount of gluten in spaghetti decreases the amount of residue in the cooking water (Matsuo & Irvine, 1970). During cooking, a weak or discontinuous protein matrix permits exudates to escape during starch granule gelatinisation into the cooking water (Sharma *et al.*, 2002; Marconi *et al.*, 2000).

3.2.4.4. Acidity of cooking water

ANOVA test showed that the type of macaroni, storage period and cooking time significantly ($P<0.05$) affected the acidity of the cooking water. It was more convenient to measure acidity of cooking water rather than macaroni to evaluate simultaneously the effect of cooking time, storage and type of macaroni.

During storage, enrichment resulted in increased acidity of the cooking water.

Duncan's multiple range test showed that RM has significantly ($P<0.05$) higher acidity than CM and MM (Table 3.11).

Microwaving the wheat germ had a significant negative effect on acidity increase. MM showed lower acidity increase when compared to RM. Also the ANOVA test showed that there is a significant interaction between storage period and type of macaroni. Storing of the macaroni samples is more effective on increasing the acidity of the cooking water. A significant ($P<0.05$) positive correlation ($r=0.912$ for initial, $r=0.941$ for 6 month storage and $r=0.825$ for 12 moth storage) was found between total solid lost and acidity of the cooking water. In all cases acidity of the cooking water was increased with increasing cooking loss. It was found that increase in the acidity of enriched samples during storage did not affect the results of sensory evaluation scores (Table 3.6).

Table 3.11. Changes in acidity (%) of cooking water at different cooking times and during storage [A]

Storage		Cooking Time (min)		
Time	Type	10	20	30
Initial	CM	0.21±0.007 (b, C)	0.36±0.014 (a, B)	0.45 ±0.014 (a, A)
	RM	0.27±0.007 (a, C)	0.37±0.007 (a, B)	0.46±0.072 (a, A)
	MM	0.28±0.002 (a, C)	0.38±0.071 (a, B)	0.48±0.072 (a, A)
6 months	CM	0.21±0.071 (b, C)	0.37±0.014 (ab, B)	0.49±0.006 (ab, A)
	RM	0.34±0.007 (c, C)	0.51±0.007 (c, B)	0.71±0.014 (c, A)
	MM	0.29±0.004 (a, C)	0.49±0.004 (c, B)	0.68±0.007 (c, A)
12 months	CM	0.22±0.014 (b, C)	0.39±0.007 (b, B)	0.52±0.023 (b, A)
	RM	0.69±0.007 (d, C)	0.78±0.006 (d, B)	1.27±0.074 (d, A)
	MM	0.47±0.007 (e, C)	0.59±0.014 (e, B)	0.93±0.008 (e, A)

[A] Figures in the same raw sharing a common capital letter in parenthesis are not significantly different at 0.05 level
Figures in the same column sharing a common a letter in parenthesis are not significantly different at 0.05 level
See Table 3.4 for abbreviations.

Wheat germ contains high amounts of unsaturated fatty acid and enzymes such as lipase and lipoxidase which may cause an increase in acidity during storage. The results were similar with the results reported by Duszkiewicz-Reinhard *et al.*, (1988).

They found that acidity of spaghetti fortified with legume flours and protein concentrates was higher and significantly different from the control.

3.2.5. Texture

Compressibility data of macaroni samples are shown in Figure 3.14. ANOVA showed that there is not a significant ($P<0.05$) difference between compressibility of the macaroni samples. It was surprising to note that all the samples have the same compressibility value. Enrichment with RG and MG had no significant effect on compressibility of macaroni. Increasing the cooking time resulted in significant ($P<0.05$) increase in compression. Studies of Rayas-Duarte *et al.*, (1996) indicated that spaghetti made from dark buckwheat and amaranth samples showed significant decrease in firmness while the spaghetti containing lupin samples showed firmness similar to the control as found in this study.

Dexter and Matsuo (1979) reported that the amount of solid lost to cooking water, the cooked weight and the strand diameter did not appear to be related to spaghetti cooking quality. They found that the superior spaghetti cooking quality might be related to the proportion of insoluble protein in cooked spaghetti. The results revealed a rapid decrease in total salt-soluble proteins (albumin and globulins) and soluble gluten proteins (gliadins and glutenins) up to about 12 min, concomitant with an increase in insoluble protein. Although the addition of wheat germ resulted in a decrease in gluten matrix, and increase in cooking loss, wheat germ contains approximately 30% insoluble protein. This may explain why there was no change between compressibility of enriched macaroni samples and control. It was also reported that spaghetti firmness, elasticity and surface characteristics improve with increasing protein content. This has been attributed to the higher number of polypeptide chains associated with higher protein levels that increase the chance for proteins to interact to form an insoluble network (Malcolmson, Marsuo & Balshaw, 1993).

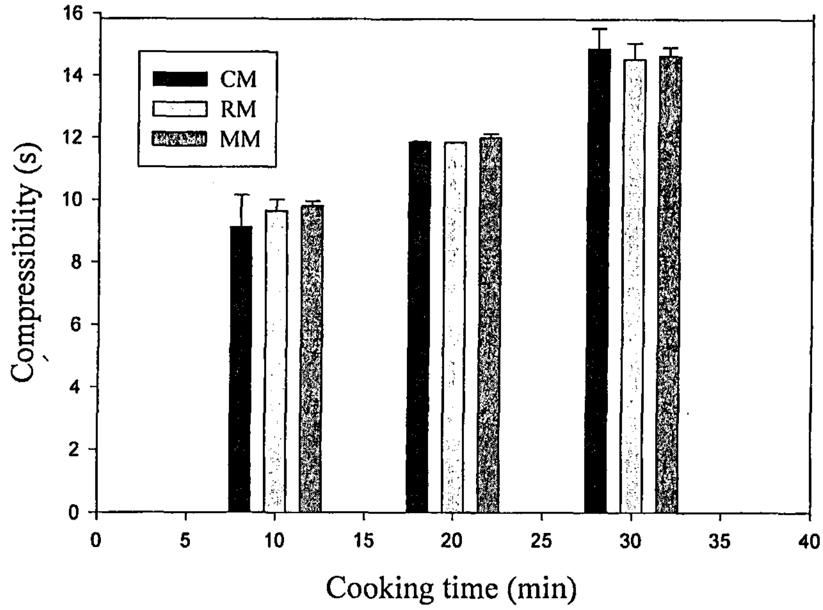

Figure 3.14. Effect of enrichment and cooking time on compressibility of macaroni
See Table 3.4 for abbreviations

3.2.6. *In-vitro* Protein Digestibility

The characteristics often used to determine the protein quality of a food are its amino acid composition, bioavailability and protein digestibility (Maha, Abdullahi & Abdelwahab, 2003; Dahlin & Lorenz, 1993). The protein digestibility is a primary determinant of the availability of amino acids and therefore protein digestibility is important when evaluating the nutritive quality of food protein (Habiba, 2002).

Uncooked enriched macaroni samples were evaluated for in vitro protein digestibility (IVPD) in comparison to durum wheat macaroni (control) over a year of storage. Data obtained about the IVPD of the uncooked macaroni samples used in this study were used mainly as a basis for comparison when assessing digestibility improvements after enrichment of the macaroni with raw and microwaved wheat germ. Heating may improve the digestibility of proteins by denaturating the protein, exposing new sites for enzyme attack. Heating also aids protein digestibility by inactivating enzyme inhibitors (Dahlin & Lorenz, 1993). Rayas-Duarte *et al.*, (1996), Dahlin and Lorenz (1993), and Hsu *et al.*, (1977), all used uncooked samples to determine the IVPD of pasta, as we have in this study.

The IVPDs of the macaroni samples were monitored over 0, 6, and 12 months_of storage by measuring the pH drop of the medium over the ten minute period after the addition of trypsin enzyme (Fig. 3.15). As shown in Fig. 3.15, the pH drops suddenly for the first two minutes and then decreases more slowly after 4 min incubation as the substrate to enzyme ratio declines. During cereal-enzyme incubation, hydrolysis occurs and amino acids are released from the peptide chain. This results in decreased pH, a marker for increased protein digestibility (Dahlin and Lorenz, 1993).

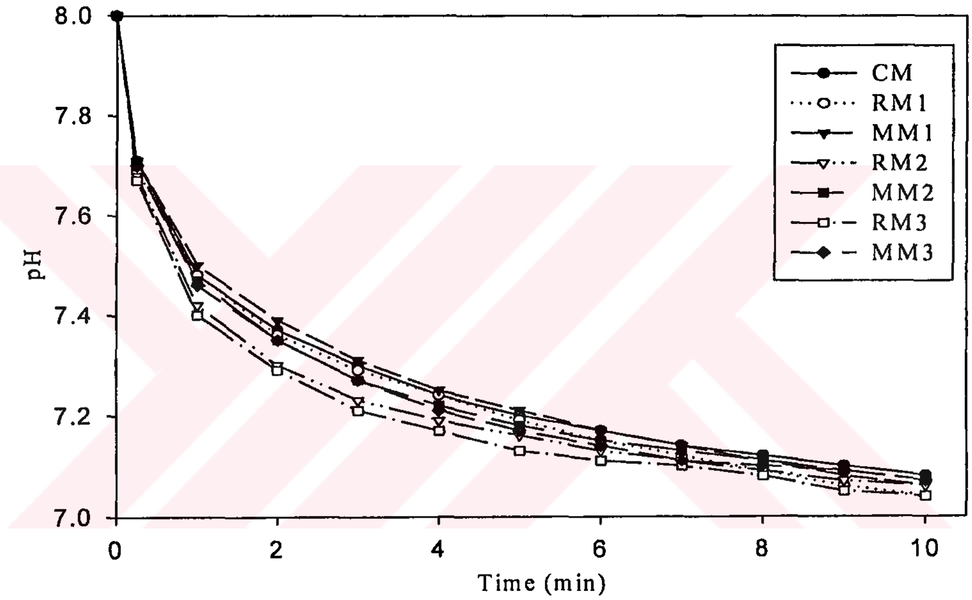

Figure 3.15. The pH drop of macaroni samples during IVPD measurements (1=initial values, 2=after six month storage, 3=after twelve moth storage)
See Table 3.4 for abbreviations

Rayas-Duarte *et al.*, (1996) found similar results, that spaghetti made from lupin flours retain their overall digestibility compared to the controls. They found that the IVPD of the control was around 85.5%, which was similar to our control results.

The ANOVA test showed that there was no significant (P<0.05) enrichment effect with RG and MG on the IVPD of macaroni over the year in storage when compared to CM (Table 3.12). Wheat germ containing macaroni samples appeared to retain their overall protein digestibility, around 82.8%, compared to 82.7% for the control. These results may be explained by a possible lack of antinutrients (enzyme inhibitors) in wheat germ.

Table 3.12. IVPD of macaroni samples (%) after different storage periods [A]

Type	Initial	6 month	12 month
CM	82.4±0.12 (a, A)	82.6±0.13 (a, A)	82.3±0.00 (a, A)
RM	82.9±0.25 (a, A)	82.7±0.25 (a, A)	82.8±0.39 (a, A)
MM	82.7±0.00 (a, A)	82.5±0.00 (a, A)	82.6±0.00 (a, A)

[A] Means with the same grouping letter in the row are not significantly different (α=0.05) Means with the same grouping letter in the column are not significantly different (α=0.05)
See Table 3.4 for abbreviations.

3.2.7. Differential scanning calorimetry

The objective of the DSC measurements was to determine changes in starch gelatinization after addition of raw and microwaved wheat germ to macaroni, since wheat germ contains high amounts of protein and fat, and to correlate starch interaction properties with pasta cooking quality (pasta cooking quality has been described as a competition between starch gelatinization and protein network formation).

In the literature, DSC studies of macaroni have usually been used to predict the changes in starch gelatinization under different drying conditions Yue, Rayas-Duarte and Elias (1999); Zweifel *et al.*, (2000) and Güler *et al.*, (2002); Riva, Fessas, Schiraldi (2000). Some authors have also studied the effects of lipid and protein-starch interactions using DSC, and they have found significant influences of lipid and protein on starch gelatinization (Mohamed and Rayas-Duarte, 2003; Villwock, Eliasson, Silverio, BeMiller, 1999).

In the present study the thermal properties of semolina and control macaroni (dry) were compared with semolina blends (semolina mixed with raw and microwaved wheat germ) and enriched macaroni samples.

DSC results showed that there are two distinct, symmetrical endothermic transitions.

The first can be attributed to the gelatinization of starch at excess water (P1), and second is the reversible dissociation of preexisting amylose-lipid complexes. Yue *et al.*, (1999); Zweifel *et al.*, (2000); and Güler *et al.*, (2002) found two similar endothermic peaks to the ones we found in this study. Thermograms for them are shown in Fig. 3.16.

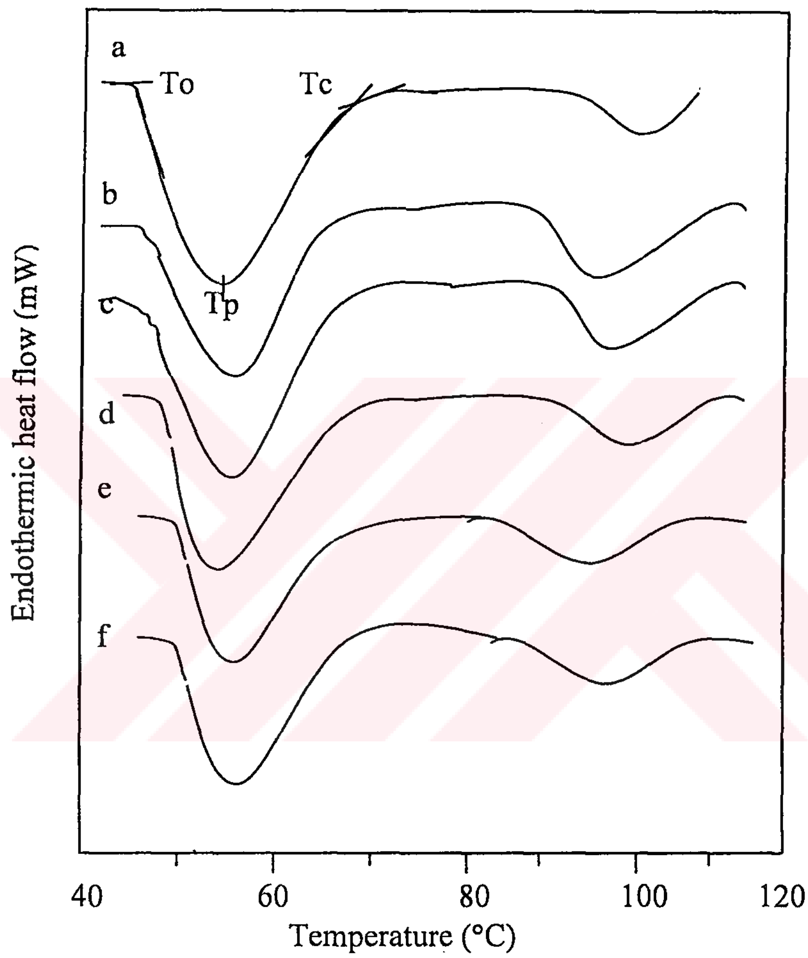

Figure 3.16. Differential scanning calorimetry thermograms of semolina (a), semolina blended with 15% raw wheat germ (b), semolina blended with 15% microwaved wheat germ (c), CM (d), RM (e), MM (f)
See Table 3.4 for abbreviations

The first melting transition of the samples is represented in Table 3.13. In general, the temperatures of gelatinization onset (T_{o1}), the melting temperatures (T_{p1}), the melting temperature ranges (ΔT_1), and melting enthalpy values (ΔH_1) of the semolina and macaroni produced from 100% semolina (the control) are in good agreement with the values obtained by Zweifel *et al.*, (2000) and Güler *et al.*, (2002), but significantly lower than those obtained by Yue *et al.*, (1999). The differences may be due to variations between starches in samples.

T_{o1} and T_{p1} values for the control macaroni were shifted to higher values than for the semolina. The shift in T_{p1} represented a decrease in the gelatinization semolina, and the range ΔT_1 was reduced by approximately 3.2 °C for the control_enthalpy of the macaroni compared to the semolina, from 5.47 to 4.4 J/g (Table 3.13). Therefore macaroni samples had narrower gelatinization temperature ranges, and lower enthalpies than semolina, indicating that the starch molecules had more mobility and underwent conformational reorganization during the drying period of the macaroni. The increase in the gelatinization temperature (T_{o1} and T_{p1}) observed in the macaroni samples may show stabilization of amylopectin, which forms crystallites (Yue *et al.*, 1999). Larsson and Eliasson (1991) reported that a decrease in the melting range of pasta starch might be due to greater uniformity among crystalline species made of amylopectin. It was also stated that during the drying of the pasta, more stable homogenous microcrystalline species gelatinize and consume less energy (Yue *et al.*, 1999).

Table 3.13. Gelatinization characteristics of samples obtained from first endothermic peak of DSC[A]

Type	To_1 (°C)	Tp_1 (°C)	ΔT_1 (°C)	ΔH_1 (J/g)
Semolina (S)	53.2±0.01 (f)	58.88±0.01 (f)	12.54±0.12 (a)	5.47±0.02 (a)
S and RG	54.9±0.01 (d)	59.2±0.01 (d)	10.4±0.02 (c)	4.6±0.02 (c)
S and MG	54.2±0.02 (e)	59.1±0.01 (e)	11.1±0.02 (b)	4.9±0.05 (b)
CM	55.6±0.05 (c)	60.4±0.05 (c)	9.4±0.06 (d)	4.4±0.02 (d)
RM	56.5±0.03 (a)	60.5±0.02 (b)	8.6±0.03 (f)	4.0±0.02 (f)
MM	56.1±0.02 (b)	60.6±0.02 (a)	9.0±0.03 (e)	4.1±0.02 (e)

[A] Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level
T_o = Onset temperature, T_p = Peak temperature, ΔT = Melting ranges, ΔH = Enthalpy
See Table 3.4 for abbreviations.

Addition of raw (RG) and microwaved (MG) wheat germ to semolina significantly increased the values of T_{o1} and T_{p1} and reduced ΔH_1 (Table 3.13). This may be explained by the protein content of wheat germ, which contains soluble gluten proteins, gliadins and glutenins. Studies showed that the gelatinization peak temperature of the starch increased and ΔH decreased in the presence of gluten proteins. The ΔH of gelatinization decreased as the amount of gluten proteins increased, while the peak temperature increased as the ratio of the protein starch increased (Eliasson, 1983). Mohamed and Rayas-Duarte (2003) reported that a decrease in ΔH values could mean that the starch in the blend has less available water due to protein competition. Higher onset and peak temperatures lowered the ΔH values, indicating incomplete gelatinization. It was found that water migration between the starch and the protein is shown to have major effect on starch gelatinization (Mohamed and Rayas-Duarte, 2003). Results showed that the semolina blend prepared from RG increased T_{o1} and T_{p1} and reduced ΔH_1 much more than the blend prepared from MG. This may be due to the effect of microwave heating on the structure of wheat germ.

Enriched macaroni samples have higher T_{o1}, T_{p1} and lower ΔH_1 and ΔT_1 compared to control macaroni (Table 3.13). Thermal changes on starch gelatinization were higher in macaroni produced with raw wheat germ. These differences may be due to the protein content of wheat germ as explained above.

Data relating to the second endothermic peak, which represents dissociation of the amylose lipid complex, are shown in Table 3.14. Generally, melting started at 89–92.9 °C and peaked at approximately 97 °C. The results were similar to those described by Yue *et al.*, (1999) and Zweifel *et al.*, (2000). There is a significant difference between the ΔH_2 values of the samples. Overall, the ΔH_2 values of the samples were significantly higher than for the semolina. The second enthalpies (ΔH_2) of the semolina and control macaroni were 4.4 and 2.3 times lower than their initial gelatinization enthalpies respectively. Addition of raw and microwaved wheat germ significantly increased the ΔH_2, ΔT_2 and reduced T_{p2} values of semolina and macaroni. It was found that raw wheat germ was more effective than microwaved wheat germ at producing starch gelatinization of semolina and macaroni. Melting ranges of semolina blends were significantly broader than semolina and macaroni samples. Overall, the ΔH_2 values of control and enriched macaroni samples were significantly higher than semolina, indicating that there were amylose-lipid inclusion complexes. Higher melting ranges, peak temperatures and melting enthalpies of semolina blends and enriched macaroni samples than semolina and control macaroni, may be explained by higher lipid content and wheat germ composition. Wheat germ contains 20.4% lipids and it increased the lipid content of the macaroni from 2.3 to 5.2%. With the addition of wheat germ, the amount of amylose-lipid inclusion complexes may be increased. It was found that the thermogram of common corn starch obtained from the starch-lipid complex becomes larger as surfactant is added (Kim & Walker, 1992). When the starch was defatted before DSC, the largest peak in the thermogram of ae du maize disappeared (Villwock *et al.*, 1999). They also found that the nature of starch-lipid complexation varied considerably depending on the nature of the lipid and the structural arrangement of amylose and amylopectin within the granule.

Table 3.14. Second endothermic transition of samples obtained from DSC[A]

Type	To_2 (°C)	Tp_2 (°C)	ΔT_2 (°C)	ΔH_2 (J/g)
Semolina (S)	92.96±0.02 (a)	97.54±0.02 (b)	9.5±0.05 (f)	1.23±0.03 (f)
S and RG	90.08±0.02 (d)	97.25±0.04 (c)	15.75±0.03 (a)	2.62±0.02 (c)
S and MG	90.65±0.05 (c)	97.03±0.02 (d)	14.59±0.04 (b)	2.063±0.02 (d)
CM	90.85±0.03 (b)	97.65±0.03 (a)	12.59±0.04 (e)	1.99±0.03 (e)
RM	89.05±0.04 (e)	97.05±0.03 (d)	14.21±0.05 (c)	2.96±0.02 (a)
MM	89.55±0.03 (f)	97.08±0.02 (d)	13.89±0.06 (d)	2.84±0.02 (b)

[A] Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level
See Table 3.4 and Table 3.13 for abbreviations.

From these results, it is obvious that the starch properties of raw and microwaved wheat germ were significantly affected the properties of macaroni in varying degrees. As explained before pasta cooking quality, such as weight and volume increase, and cooking loss was significantly affected by raw and microwaved wheat germ addition, and that macaroni cooking time was reduced from 10 min to 9 min by the enrichment. But the results showed that the compressibility of enriched macaroni was not significantly different from the compressibility of control macaroni . When these results were compared with the results from the DSC study, it was concluded that protein is the primary factor that affects the quality of macaroni, but changes in the starch properties with the addition of raw and microwaved wheat germ may also affect the pasta cooking quality.

3.2.8. Microbial count

Macaroni is generally regarded as a microbiologically safe product, as it has a relatively low water activity. Wheat germ is a by-product of wheat, which may be contaminated by pathogens, which can then survive for extended periods. Therefore, in this study, aerobic plate counts (APC), as well as mold and yeast counts were conducted for the control and enriched macaroni samples over the year of storage and the results were compared with the standards given by the food codex (Anonymous, 2001).

The results showed that the APCs of both samples were not significantly (P<0.05) different from each other (Table 3.15), and no significant increase was found over the year of storage.

Table 3.15. Aerobic plate count of the macaroni samples (log of cfu/g)[A]

Storage Period	CM	RM	MM
Initial	2.33±0.00007 (a, A)	2.33±0.00014 (a, A)	2.33±0.00007 (a, A)
6 months	2.33±0.00007 (a, A)	2.33±0.00007 (a, A)	2.33±0.00007 (a, A)
12 months	2.33±0.00014 (a, A)	2.33±0.00007 (a, A)	2.33±0.00007 (a, A)

[A] Means with the same grouping letter in the row are not significantly different (α=0.05) Means with the same grouping letter in the column are not significantly different (α=0.05)
See Table 3.4 for abbreviations.

At first, the mold and yeast counts of the enriched macaroni samples were slightly higher than those of the control (Table 3.16). Statistical analysis of the data showed that the mold and yeast counts of both macaroni samples increased significantly (P<0.05) during storage, and a higher increase was obtained in macaroni with raw wheat germ. Studies made by Berghofer et al., (2002) indicated that wheat germ has a higher amount of aerobic bacteria, mold and yeast than other outer grain fractions. They also showed that viable aerobic mesophilic counts, and mold and yeast counts were often lower in flour, bran, and wheat germ when compared to whole wheat. The mode and maximum counts for all microorganisms were decreased by one to three log units by the milling stages.

According to macaroni standards (Anonymous, 2001), APC, mold, and yeast counts for macaroni samples should not be greater than 5 and 2.699 log units/g macaroni respectively. The results show that all macaroni samples studied were in the safe limit described by (Anonymous, 2001).

Table 3.16. Mold and yeast count of the macaroni samples (log of cfu/g)[A]

Storage Period	CM	RM	MM
Initial	1.73±0.00035 (b, C)	1.74±0.00140 (a, C)	1.74±0.00035 (a, C)
6 months	1.77±0.00035 (c, B)	1.79±0.00014 (a, B)	1.78±0.00035 (b, B)
12 months	1.79±0.00007 (c, A)	1.85±0.00071 (b, A)	1.83±0.00035 (b, A)

[A] Means with the same grouping letter in the row are not significantly different (α=0.05) Means with the same grouping letter in the column are not significantly different (α=0.05)
See Table 3.4 for abbreviations.

CHAPTER IV
CONCLUSIONS

1. The oxidative stability of whole wheat germ was found as 34, 22, 12 day at 60, 70 and 80 °C respectively. The activation energy of whole wheat germ was 51.13 kJ/mol.

2. Addition of 15% wheat germ increased the protein content of control macaroni (produced from 100% drum wheat) from 11.4 to 13.3 %.

3. Based on sensory tests it was found that there was significant ($P<0.05$) difference between control and enriched types of macaroni in flavor and appearance (degree of difference was found to be slight) but not in texture and overall score. Storage of macaroni samples for one-year did not change the sensory properties significantly ($P< 0.05$).

4. Optimum cooking time of macaroni decreased from 10 min to 9 min with the addition of raw and microwaved wheat germ.

5. During cooking, percent weight increase of control macaroni decreased from 180.2 % to 174.2 and 154.8 % with the addition of raw and microwaved wheat germ respectively. Significant increase in the weight of macaroni samples was obtained as the cooking time increased. Storage of macaroni samples up to one-year did not significantly affected results of the weight increase.

6. Percent volume increase (swelling) of macaroni samples was significantly

affected by cooking time and wheat germ addition. Whereas one year storage period had no significant affect on swelling.

7. Cooking losses significantly increased with addition of wheat germ.

8. There was no significant difference between compressibility of macaroni samples of RM, MM and C. Increasing the cooking time resulted in significant increase in the compressibility of macaroni samples.

9. There was no significant effect of enrichment and storage through one year on in-vitro protein digestibility (IVPD) of macaroni.

10. DSC measurements indicated that addition of wheat germ into semolina significantly increased T_{01} and T_{P1} of macaroni samples. Enriched macaroni samples have higher T_{O1}, T_{P1} and lower ΔH_1 and ΔT_1 when compared to control macaroni.

11. Aerobic plate count (APC) of macaroni samples was not significantly different from each other and there was no significant increase was obtained during one-year storage. Mold and yeast count of enriched macaroni samples was significantly higher than control macaroni and significant increase was observed during one-year storage. Both APC and mold and yeast count of macaroni samples were after one year storage in the limits given by food codex.

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APPENDICES

Table A.1. Questionnaries used for triangle test

PRODUCT:

Two of these samples are identical, the third is different.

1. Taste the samples in the order identicated and identify the odd sample

Code	Check the odd sample
346	________________
859	________________
714	________________

2. Indicate the degree of difference between the duplicate samples and the odd sample

   Slight ____________

   Moderate ____________

   Much ____________

   Extreme ____________

3. Acceptibility:

   Odd sample more acceptable ____________

   Duplicates more acceptable ____________

   Both are same ____________

4. Comments:

Table A.2. Questionnaries used for hedonic scale

Appearance

1 2 3 4 5 6 7

Pale Normal Too Dark

Texture

1 2 3 4 5 6 7

Soft Normal Too firm, chewy

Flavor

1 2 3 4 5 6 7

None Normal Too strong

Over-all Score

1 2 3 4 5 6 7

Dislike Like very much

Which sample is the best?--------------------------------, The worst?-----------------

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