

**Effect of Processing on The Selected Properties of
Cowpea Flour To Be Incorporated Into Spaghetti**

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ABSTRACT

EFFECT OF PROCESSING ON THE SELECTED PROPERTIES OF COWPEA FLOUR TO BE INCORPORATED INTO SPAGHETTI

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In this study, various types of cowpea flours were produced from germinated, fermented and cooked cowpea or cowpea flour separately to decrease the antinutritional factors and obtain a more beneficial product. The untreated cowpea flour was also produced. These four types of cowpea flours were used in the enrichment of spaghetties at different levels (10, 15, 20, 25% w/w). The spaghetti samples were stored at room temperature (~25°C) for 12 months. Some selected properties of the cowpea flours and spaghetti samples, such as ash, moisture, protein, oil contents and color values were determined. The phytic acid content and total antioxidant capacity of the spaghetti samples were determined initially, after six months and twelve months of storage. The spaghetti samples supplemented with 20% cowpea flours were also analysed for *in vitro* protein digestibility (IVPD), microbial count (total bacteria, mould and yeast) and gelatinization behaviour over the storage period at room temperature.

Addition of cowpea flour increased the protein content of spaghetti up to 15.29% (db) from 11.73% (db). The amino acid profile of the spaghetties was also improved based on the knowledge that the best amino acid profile for a cereal product was obtained by combining them with legumes. The sensory test results of the spaghetties enriched with cowpea flour up to 25% were not different from that of the control spaghetti generally, with regard to stickiness, bulkiness and firmness. The spaghetties

enriched with the fermented cowpea flour had significantly higher “total organic matter” (TOM) values. The enriched spaghetti samples were darker in colour than the control spaghetti sample. Cooking had a negative effect on the total antioxidant capacity (TAC) of cowpea flours while germination increased the TAC of the samples. There was a slight decrease in the TAC values of spaghetti samples during the storage period. While, the cowpea flour supplement caused an increase in the phytic acid content of the spaghetti samples, the decrease in the phytic acid content with respect to time was about 0.3% for six months period and 1% for twelve months period and was not significantly important for most of the samples ($p>0.05$).

The spaghetti samples were analysed using differential scanning calorimetry (DSC) to determine the gelatinization behaviour of starch as a component of them. Two endothermic peaks were observed with significant differences in the gelatinization enthalpies (ΔH) of control and cowpea added spaghetti samples. The transition peak (T_p) temperatures were in the range of 66.9-67.9°C and 86.9-100.4°C for the first and second peaks, respectively. The transition enthalpies (ΔH) were in the range of 2.41-4.21 J/g and 1.71-3.86 J/g for the first and second peaks, respectively. The cowpea flour addition decreased the enthalpy values for starch gelatinization and increased it for protein denaturation. Totaly the heat needed for the cooking of spaghetti increased by the enrichment with the exception of cooked cowpea flour added samples that need equal amount of heat with the control spaghetti.

Supplementing spaghetti with the cowpea flour did not have a significant effect ($p>0.05$) on *in vitro* protein digestibilities. Though there was a slight variation in aerobic plate count (APC), and mold and yeast counts of cowpea added samples ($p<0.05$); spaghetti samples were found to be safe in terms of microbiological quality after one year of storage. The best types and amount of cowpea flour to be incorporated into spaghetti were proposed to be the unprocessed and than germinated types up to 20% according to TOM values and sensory results.

Key Words: Cowpea; Differential scanning calorimetry; Fermentation; Germination; Phytic acid; Protein digestibility; Spaghetti; Starch gelatinization; Total antioxidant capacity.

ÖZET

SPAGETTİ ÜRETİMİNDE EKLENMEK ÜZERE KULLANILAN BÖRÜLCE UNUNUN BAZI ÖZELLİKLERİNE DEĞİŞİK İŞLEMLERİN ETKİLERİ

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Bu çalışmada, börülce unu veya börülcenin antibesinsel faktörlerin azaltılıp daha faydalı bir hale getirmek amacıyla ayrı ayrı çimlendirilmesi, fermente edilmesi ve pişirilmesiyle çeşitli tiplerde börülce unları üretilmiştir. İşlem görmemiş börülce unu da üretilmiştir. Bu dört çeşit börülce unları, ağırlıkça değişik oranlarda (%10, 15, 20, 25, w/w) ilave edilerek spagetti örneklerinin zenginleştirilmesinde kullanıldı. Makarna örnekleri oda sıcaklığında (~25°C) 12 ay süreyle muhafaza edildi. Börülce unlarının ve katkılı makarnaların kül, nem, protein, yağ içerikleri ve renk değerleri gibi belli özellikleri belirlendi. Spagetti örneklerinde fitik asit içerikleri, toplam antioksidan kapasiteleri ve renk değerleri gibi belli bazı özellikleri belirlendi. Makarnaların fitik asit miktarları ve toplam antioksidan kapasiteleri, başlangıçta, 6 ay ve 12 aylık muhafaza sonrasında belirlendi. % 20 oranında börülce unu katılarak üretilen makarna örnekleri oda ısısındaki muhafaza süreçlerinde *vitro*da protein sindirilebilirliği (IVPD), mikrobiyolojik (toplam bakteri, küf ve maya) ve jelatinizasyon özellikleri bakımından da analiz edildi.

Börülce unu ilavesi makarnanın protein içeriğini %11.73 (kb)'den %15.29 (kb)'a kadar arttırdı. Makarnaların amino asit profilleri de tahıllarda en iyi amino asit profilinin baklagillerle kombine edilmesiyle elde edildiğine dair olan bilgiye dayanarak geliştirildi. Yapışkanlık, kümeleşme ve sertlik kriterleri gözönüne

alındığında genel olarak, %25'e kadar brlce unu katkılı makarna rneklerinin duyusal test sonuları katkısız makarnaya gre farklı bulunmadı. Fermente edilmiř brlce unu katkılı makarnalar anlamlı olarak daha yksek "toplam organik madde" (TOM) deęerine sahip oldu. Katkılı makarnalarda kontrol makarnasına gre daha koyu bir renk tesbit edildi. Piřirme, toplam antioksidan kapasiteyi (TAC) olumsuz ynde etkilerken, imlendirmeyele rneklerdeki TAC deęerlerinde artıř grld. Muhafaza srecinde, makarna rneklerinin TAC deęerlerinde bir miktar dřř gerekleřti. Brlce unu katkısı makarnaların fitik asit ierięinde artıřa neden olurken, fitik asit oranlarındaki zamana baęlı azalma altı aylık srete %0.3 ve 12 aylık srete %1 civarında olup, bir ok rnekten anlamlı bulunmadı ($P>0.05$).

Makarna rnekleri, ieriklerinde bulunan niřastanın jelleřme zelliklerinin belirlenmesi amacıyla diferansiyel tarayıcı kalorimetresi (DSC) ile analiz edildi. Kontrol ve katkılı makarna rnekleri iin, jelatinizasyon entalpilerinde (ΔH) anlamlı farklılık olan iki endotermik geiř gzlendi. rneklerin geiř pik sıcaklıkları (T_p) birinci ve ikinci piklerde sırasıyla 66.9-67.9°C ve 86.9-100.4°C aralıklarındaydı ve geiř entalpi (ΔH) deęerleri de birinci ve ikinci piklerde sırasıyla 2.41-4.21 J/g ve 1.71-3.86 J/g aralıklarındaydı. Brlce unu katkısı niřasta jelatinizasyonu iin entalpi deęerini dřrrken, protein denatrasyonu iin entalpi deęerini ykseltmiř, kontrol makarnasıyla eřit miktarda ısı gerektiren kaynatılmıř brlce unu ilaveli makarna rneęi hari, brlce unu ilavesi makarnaların piřmesi iin gerek duyulan ısıyı toplam olarak artırmıřtır.

Brlce unu katkısının, makarnaların *vitro*da protein sindirilebilirlięini etkilemedięi, toplam bakteri yk ve toplam kf ve maya sayılarında bir miktar deęiřme olmakla birlikte bir yıl sonunda makarna rneklerinin tketiminin gvenli olduęu bulunmuřtur. TOM deęerleri ve duyusal sonulara dayanarak, makarnaya ilave edilecek en iyi brlce unu tipi ve oranı herhangi bir iřlem grmemiř ve daha sonra imlendirilmiř brlce unu olarak ve %20 ye kadar belirlendi.

Anahtar Kelimeler: Brlce; Diferansiyel tarayıcı kalorimetresi; Fermantasyon; imlendirme; Fitik asit; Protein sindirilebilirlięi; Spagetti; Niřasta jelatinizasyonu; Toplam antioksidan kapasite.

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1. INTRODUCTION

1.1. Cowpea Plant

Cowpea (*Vigna unguiculata* or *V. sinensis*) sold under such names as field peas, southern peas, crows and black-eyed peas is one of the important food legumes and a valuable component of the traditional cropping systems in the semi-arid tropics covering Asia, Africa, Central and South America (Prinyawiwatkul et al., 1996a). Being a drought tolerant and warm weather crop, cowpea is well-adapted to the semi-arid regions of the tropics where other food legumes do not perform well. Cowpea has the unique ability to fix atmospheric nitrogen through its nodules and grows well even in poor soils with more than 85% sand, less than 0.2% organic matter and low levels of phosphorus (Kolawale et al., 2000). Also, it is shade tolerant and, therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton as well as with several plantation crops (Singh et al., 2003). Cowpea serves as a key staple food for many developing countries, providing more than half the plant protein in the human diet. Based on the FAO data (<http://www.fao.org>) the estimated worldwide area under cowpea is about 14 million ha, with over 4.5 Mt annual production.

1.2. Health Benefits

Beans have long been recognized as a valuable source of plant protein, vitamins (thiamine and niacin), minerals (P, K, Ca, Mg) and dietary fiber. There is considerable evidence in the literature that foods high in water-soluble dietary fibre (such as bean products) and purified forms of water soluble dietary fibre can reduce blood cholesterol (Uebersax et al., 1991). Bean products have been shown to be able to lower serum cholesterol levels. Daily consumption of 100-135 gr of dry beans reduced serum cholesterol levels by ~20% thereby reducing the risk of coronary hearth disease by 40% (Anderson, 1985). Nutritional therapy combining high-fiber diets with bean supplementation is well tolerated and associated with no major side effects, except for a reported increase in flatulence and eructation. Besides being a good source of both soluble and insoluble dietary fiber and possessing the health-

related benefits associated with both types of dietary fiber, beans have an added advantage of being an inexpensive fiber source. Beans are low in fat (1-3%) and high in complex carbohydrates (50-60%). These characteristics make beans ideally suited to helping consumers meet the dietary goals of reducing fat intake and increasing the intake of starch and other complex carbohydrates (Huges, 1991). Cowpea containing 24-26% crude protein and being rich in glutamic acid, aspartic acid and lysine is a nutritionally important seed (Prinyawiwatkul et al., 1996a).

1.3. Chemical Composition

The proximate composition data for the dry cowpea seed is summarized in Table 1.1. These values varies due to genetic background, agronomic practices, postharvest handling and storage, age of seeds, and processing treatments prior to food preparations (Uzogara and Ofuya, 1992).

Table 1.1. Composition of whole dry cowpea (based on 8% moisture) (Phillips and McWatters, 1991)

Component	Amount/100 gr	Component	Amount/100 gr
Crude Protein (Nx6.25)	24 gr	Calcium	110.0 mg
Carbohydrate	63 gr	Copper	0.8 mg
Total Dietary Fiber	2.7 gr	Iron	8.3 mg
Crude Fat	1.9 gr	Magnesium	184.0 mg
Energy	340 kcal	Phosphorus	425.0 mg
Thiamin	0.9 mg	Potassium	1,110.0 mg
Riboflavin	0.2 mg	Selenium	84.5 mg
Niacin	2.0 mg	Sodium	16.2 mg
Pyridoxine	0.4 mg	Zinc	3.4 mg
Folacin	0.6 mg		
Pantothenic acid	1.5 mg		
Vitamin C	1.5 mg		
Vitamin A	5.0 RE		

As in the other legumes, proteins in cowpeas are rich in glutamic acid, aspartic acid, and lysine but low in sulfur amino acids. Methionine is the first limiting amino acid in cowpea protein (Aremu, 1990). The high lysine content (486 mg/g N, Table 1.2) makes cowpeas an excellent enhancer of protein quality when combined with cereal grain proteins which are low in lysine but rich in sulphur containing amino acids (Phillips and McWatters, 1991). Maximum nutritional benefits are said to be achieved by complementing cereals with cowpeas at the ratio of 45:15 (cereal:cowpea, w/w), which yields amino acid scores closer to the FAO/WHO Standard (Bressani, 1985).

Table 1.2. Mean essential amino acid content of eight cowpea varieties (Prinyawiwatkul et al., 1996a)

Amino acid	Amount(mg/gr nitrogen)	Amino Acid	Amount(mg/gr nitrogen)
Arginine	500±53	Histidine	213±19
Isoleucine	318±10	Leucine	484±31
Lysine	486±11	Methionine	79±10
Cystine	32±4	Phenylalanine	263±12
Tryptophan	68±10	Valine	314±33

Phillips (1982) fractionated cowpea seeds into cotyledons and seed coat and studied their amino acid compositions. All fractions are rich in lysine, but rather severely limiting in sulfur amino acids. There is a little difference in essential amino acid profiles of cotyledon and seed coat fractions indicating that the removal of seed coat (dehulling or decortication) does not change the essential amino acid composition of the seed. The advantage of decortication lies largely in the removal of seed coat tannins, anthocyanins, and trypsin inhibitors; however, some dietary fiber, calcium, and iron may be lost through decortication (Walker, 1982).

Cowpeas are also a good source of dietary carbohydrates, containing 50 to 65%, of which starch contributes about 60 to 77% (Longe, 1980). Sucrose is the most abundant sugar, followed by stachiyose, verbascose, and raffinose. Cowpeas contain large amounts of crude fiber, of which cellulose is a major component. The carbohydrate content of cowpeas is given in Table 1.3.

Cowpeas are low in fat and contain no cholesterol. The lipid content in cowpeas ranges from 0.7 to 3.5%, and unsaturated fatty acids constitute more than two-thirds of the total fatty acids (Mahadevappa and Raina, 1978). Linoleic, linolenic, and palmitic acids are the major fatty acids in cowpea lipids. Cowpeas are an excellent source of niacin, thiamine, and other water soluble vitamins, and also essential minerals such as calcium, magnesium, potassium, and phosphorus (Phillips and McWatters, 1991).

Table 1.3. Carbohydrate content of cowpeas (% in dry weight basis)
(Prinyawiwatkul et al., 1996a)

Composition	Range
Total carbohydrates	56.0-68.0
Starch	31.5-48.0
Total soluble sugars	6.0-13.0
Oligosaccharides	
Sucrose	1.8-3.1
Raffinose	0.4-1.2
Stachyose	2.0-3.6
Verbascose	0.6-3.1
Crude fiber	1.7-4.0

1.4. Antinutritional Factors

The nutritional value of food grain legumes can be divided into two large groups as positive and negative factors. As positive factors, for population living in developing countries, food grain legumes represents foods that provide protein not unlike what meat and cheese do for those in the developed world. Because of their high protein and lysine content, they also represents good sources of supplementary protein when added to cereal grains and root crops, which are low in protein and lysine. Furthermore, grain legumes contain high levels of dietary fiber and phytochemicals, which may be closely associated with health promotion. However, the acceptability

of and use of food grain legumes has been limited by the presence of antinutritional factors.

Despite its high protein content, cowpeas have some limiting amino acids and contain trypsin and chemotrypsin inhibitors responsible for reducing the digestibility of protein by inhibiting protease activity. Cowpeas contain, polyphenolic compounds, tannins, lectins, phytic acid and indigestible oligosaccharides.

Among the oligosaccharides raffinose, stachyose, and verbascose are not utilized by monogastric animals including humans, who lack the specific α -galactosidase enzyme needed to digest them (Brune et al., 1991). So, the use of cowpea as a food source has not been utilised to its full potential. Research has shown that some processes like germination (Nnanna et al., 1990), fermentation (Zamora and Fields, 1979) and cooking (Prinyawiwatkul et al., 1996a) of cowpeas do not only improve nutritional quality, flavor and functional properties but also reduce these undesirable factors.

Generally, phenolic compounds are chemical substances with one or more aromatic rings, bearing hydroxyl groups in different patterns. The presence of phenolic compounds, particularly condensed tannins, has been associated with lower bioavailability of minerals, proteins and carbohydrates. These compounds form complexes with food nutrients, making the latter either less soluble or less susceptible to enzymatic degradation and thus less available for absorption. The major phenolic structures with antinutritional properties include configurations such as catechol, resorcinol and galloyl rings that totally exist in cowpea grains as the amount of 9.5 mg/g cowpea grain. These phenolic groups of cereal and legume grains may have different antinutritional effects depending on their structure and may be affected differently by food processing methods. Galloyl and catechol phenolics have been shown to impair iron availability in plant foods (Brune et al., 1991). These factors negatively affect the nutritive value of beans through direct and indirect reactions: they inhibit protein and carbohydrate digestibility, affecting metabolism; inhibit a number of enzymes and bind nutrients making them unavailable (Bressani, 1993).

Phytic acid (myoinositol, 1,2,3,4,5,6, hexakis-dihydrogen phosphate) exists in legumes, cereals and oil seeds naturally. Phytic acid which makes complexes with minerals like Zn, Fe, Ca, Mg, Cu and with proteins directly or indirectly changing their solubility, functionality and digestibilities is known as an antinutritional factor (Reddy and Salunkhe, 1981).

Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. In cereals 50-80% of total phosphorus, in legumes 80% of total phosphorus exists as phytate phosphorus (Lolas et al., 1976). The phytate content in cereals, legumes and oil seeds are 0.50-1.89%, 0.40-2.06% and 2.0-5.2% respectively (Yilmaz and Ünal, 1993). The phytic acid content of cowpea flour obtained in previous studies ranged from 290-992 mg/100 g (Lasztity and Lasztity, 1990; Kumar and Venkatasgmun, 1978; Ene-Obong, 1995; Giami et al., 2001).

The amount of phytic acid shows significant variations among the varieties (Lasztity and Lasztity, 1990). In a study, average phytic acid value was given as 1.37% for cowpea (Ologhobo and Fetuga, 1984). Theoretically, there are three ways in decreasing the phytate phosphorus and also other antinutritional factors in cereals and legumes: The first one is to improve the types of them by genetic modifications; the second one is to apply some processes like fermentation, germination, cooking, and extrusion; the third one is to use some methods like mechanical separation or extraction to separate them (Cheryan, 1980). Because most of the phytic acid is located in the outer parts of the kernel, low extraction white flours contain low phytic acid quantities.

One of the limitations in the use of cowpeas is their undesirable cooking characteristics which prolong cooking time and demand correspondingly higher fuel inputs. The hard-to-cook defect is the failure of leguminous seeds to become soft enough to eat after cooking for a reasonable time. It is caused by storage of beans at elevated temperature and relative humidity. In addition to reduced palatability and wasting fuel, hard-to-cook defect may compromise the nutritional quality of legume seeds (Tuan and Phillips, 1991). Molina et al. (1975) found a negative relationship between storage and protein digestibility, protein efficiency ratio, and protein solubility of black bean stored at 25°C for three and six months.

Shahidi (1997) reported that some antinutrients might exert beneficial health effects at low concentration, therefore modification of processing conditions was required to remove or reduce certain unwanted components.

1.5. Cowpea Flour Production

Despite their potential as an inexpensive source protein and energy, cowpeas are underutilized in most industrialized countries largely because of their inconvenient form when used as an ingredient in food preparations (McWatters et al, 1992). Although cowpea seeds are typically consumed as a boiled vegetable alone or in combination dishes, considerable interest has developed recently in expanding usage of cowpeas in other forms (McWatters, 1990).

Some developed technologies have been documented for cowpea flour production. For instance, the double drum drier can be used to prepare quality legume flours. Removal of the seed coat from highly pigmented or dark-colored cowpea varieties is necessary prior to flour preparation to avoid black specks that are not acceptable for many uses. Pregelatinization prior to drum drying is not necessary for several applications (Beuchat et al., 1985).

Onayemi and Potter (1976) prepared high quality drum dried cowpea powders by soaking (18 h, 23°C), decorticating, wet grinding, supplementing with various levels of DL-methionine to improve the protein quality because it is well known as the first limited amino acid in cowpeas and drum drying. These flours have good storage stability at 37°C for at least 24 weeks and are convenient to use in some traditional dishes, however, they retain a slight beany flavor, which may limit their use in certain products (Okaka and Potter, 1979).

Successful performance of legume flours as food ingredients depends upon the functional characteristics and sensory quality they impart to the end products. Although a beany flavor is not desirable in the most food products including noodles, baked goods, and snack foods, beany-flavored cowpea flour is acceptable in certain traditional products such as moin-moin (McWatters, 1990). Therefore cowpea flour with reduced beany flavour would not be appropriate for use in products that are

desirable because of their beany flavour. Okaka and Potter (1979) produced cowpea flours with reduced beany flavour and reported that beaniness was decreased by soaking cowpeas in acidified water, decorticating, and blanching with 100°C steam prior to grinding and drum drying.

The availability of ready to use cowpea flours could greatly simplify and promote the use of this legume (Henshaw and Lawal, 1993). Mass production of flour from dry cowpeas is a simpler technology than that used for oilseed flour production (McWatters, 1990). The defatting step necessary for the production of peanut flour (Prinyawiwatkul et al., 1993) is not required for cowpeas because of their low fat content.

There are a lot of studies aimed at enhancing cowpea utilization through development of a mechanical process for producing a ready-to-use cowpea flour with acceptable functional, sensory, and nutritional properties. In addition to developing technologies to improve the ease and efficiency of removing the seed coat, conditions for processing and using cowpea were tried to be optimized.

It is desirable to develop processes that provide consumers with derived products with improved nutritional quality. Simple inexpensive treatments could modify legume composition. Soaking could reduce the soluble antinutrients which can be eliminated with discarded soaking solution. Thermal treatments such as cooking deactivate heat-sensitive factors like trypsin inhibitors. Germination has been suggested as an effective treatment to remove antinutritional factors from legumes and mobilizing secondary metabolites like phytates and α -galactosidase (Ibrahim et al., 2002).

1.5.1. Decortication

Many legume grains undergo dehulling prior to consumption. Dehulling or decortication refers to the removal of the outer seed coat of the legume and leads to improved appearance, texture culinary properties, and palatability (Hudda, 1983). In addition dehulling is necessary for certain food preparations. Often, traditional dehulling is a manual process that is time consuming and labor intensive involving dry grinding on a stone or in a mortar followed by winnowing or wet grinding of

soaked seeds and causing large losses due to seed breakage and powdering. According to Eastman (1980), the demand for grain legume flour will increase if the supply of flour with acceptable texture, taste and color can be guaranteed.

Although both wet- and dry-decortication techniques were investigated, the intention was to employ dry rather than wet milling to avoid microbial growth and an extensive drying process (Phillips and McWatters, 1991). Because most cowpea cultivars have a seed coat tightly attached to the cotyledon, abrasive dry milling without prior conditioning results in undesirably low yields due to the loss of soft cotyledons. In studies to improve efficiency of decortication by dry mechanical abrasion, a predecortication treatment consisting of wetting, conditioning and drying was demonstrated to be effective (McWatters, 1990). Laboratory-scale processing of cowpea flour involves the following steps: (1) increase the cowpea moisture content from 10 to 25% by weight, (2) equilibrate at ambient temperature for 30 min with occasional stirring, (3) dry hydrated seeds in a rotary forced-air dryer at 70°C for approximately 80 min to a final moisture content of 10%, (4) decorticate in an abrasive roll-over dehuller such as a rice polisher, (5) remove seed coats by winnowing, and (6) grind the seeds through a 1.0-mm opening mesh screen. Phillips et al. (1988) reported that the decortication efficiency was improved by this predecorticating, drying treatment. The yield was said to be high enough (91-94%) by this method according to the other methods (Prinyawiwatkul et al., 1996a). Meal processed by this method has been shown to retain the functional and nutritional properties essential for good-quality akara production (Phillips et al., 1998). Because of simplicity of the process, laboratory scale cowpea flour production can be expanded to a larger scale and the milling technology for processing such a flour is said to being implemented in some places (Prinyawiwatkul et al., 1996a).

The eating quality of milled cowpea products, particularly the texture, depends on the flour composition, degree of fineness of grinding and dishes. The flour was prepared by soaking cowpeas in 25°C tapwater for 18 h, drying with forced air, coarse milling, removing the seed coat by an air blast, autoclaving the flour at 121°C for 10 min, forced-air drying and grinding to a fine flour. The obtained flour was creamy-white, free flowing, and non-hygroscopic and dispersed readily in water.

Removal of the seed coat by an air blast is said to be a significant improvement over some procedures of manual wet rubbing and flotation (Okaka and Potter, 1979).

Cowpea flours produced by a simple dry-mill process (washing in water, oven drying at 50 to 55°C, and grinding) (Zamora and Fields, 1979), a spray-drying process (Sales, 1980), freeze drying process (Schaffner and Beuchat, 1986), and for other food applications have been described.

Several researches have investigated wet and dry milling technologies to eliminate the laborious and time consuming nature of seed coat removal and wet grinding. Reichert and Youngs (1976) suggested that abrasive mills be used for dry dehulling of legume seeds; however, the yields of usable material are undesirably low. Phillips (1982) prepared flour from a type of cowpea which has a smooth, brittle, loosely adhering seed coat by cracking and winnowing techniques. This milling process resulted in 88% yield of flour from the starting cowpea seeds.

Reichert et al. (1979) examined village-scale mechanical decortication of cowpeas using wet and dry techniques. Dry decortication resulted in considerably lower (73 to 80%) yield levels compared to that (90 to 96%) from wet decortication using a rubber-matted barley deawner. However, the moisture content of the wet-decorticated products was 20 to 30% as a result of soaking step, necessitating subsequent drying. Ningsanond and Ooraikul (1989) compared dry and wet processes for milling red cowpeas. At optimum conditions, dry decortication with an abrasive device gave 75% yield of decorticated seeds with 0% of the hull remaining and 18% cotyledon loss, compared with 70% yield, 2% of the hull remaining, and 20% cotyledon loss from wet decortication of soaked seeds (8 to 10 h at 30°C) using a stone mill with a 3.5-mm clearance. There was little difference in chemical composition between flour made from both methods (Ningsanond and Ooraikul, 1989). Problems experienced with dry milling of cowpeas include inconsistent yield, incomplete separation of hull, dark color of flour, and development of off-flavor when stored. However, dry milling may be preferred to wet milling due to lower operation costs and energy requirements, less microbial contamination, and less liquid waste (Phillips, 1982).

Although decortication leads to a faster cooking time, increased digestibility, and better texture and appearance, cowpeas are not usually decorticated before cooking unless they are intended for production of cowpea flour and/or paste for preparation of steamed and fried cowpea products. To prepare cowpea flour, decortication may not be necessary, provided that cowpea cultivars with a light-colored seed coat and little pigmentation in the hilum are employed (Prinyawiwatkul et al., 1994). Flour prepared from cream-type cowpeas that were sufficiently light in overall color could be milled without decortication. Presence of the seed coat did not adversely affect batter-handling properties and had acceptable sensory qualities for fried cowpea products (McWatters and Brantley, 1982).

1.5.2. Germination

Nutritive values of several legumes have been reported to improve as a result of germination. Vaidehi et al. (1985) prepared malt powders from germinated cowpeas and other legumes that were used for biscuit preparation. Cowpeas were soaked in tapwater for 12 h and germinated for 24 h at 25°C. The root and testa were rubbed off and cleaned seeds were dried, kilned at 60 to 70°C for 30 min, and powdered.

Although germination improves the nutritional quality of legumes by reducing antinutritional and indigestible factors, a prolonged germination period initiates metabolic processes and rootlet development, which results in undesirable compositional changes and reduces yield. Nnanna et al. (1990) reported that germination conditions may be chosen that allow significant reduction in oligosaccharide content of cowpeas without excessive rootlet formation or loss of dry matter. A temperature of 30°C and time of 24 h produced the most complete destruction of undesirable oligosaccharides with little rootlet development and little effect on protein or carbohydrate content of cowpeas, and aerobic incubation following germination proved relatively ineffective in further reducing the oligosaccharide content.

The effect of germination on antinutrients in cowpea was studied and germination caused a significant reduction in tannin contents and the percentages of reduction were 12.63 and 25.82% after 24 and 48 hour of germination, respectively. The

decrease in phytic acid and trypsin inhibitor were 17.4-22.9% and 30.99-53.56% for 24 h and 48 h germinations respectively (Ibrahim et al., 2002).

1.5.3. Fermentation

Fermentation of legumes, a traditional method of food preparation, is often employed to develop desirable characteristics such as flavor, aroma and texture (Sahte and Salunkhe, 1981). Various legumes can be fermented into nutritious products. Cowpeas are a potential alternative substrate for making fermented products in countries where soybeans are not locally available (Djurtoft, 1982). Recognising similarities in size, color and composition of soybeans, and other legume seeds, it is possible that fermented products resembling tempeh and natto (both traditionally made from soybeans) could be prepared from cowpeas (Beuchat et al., 1985).

A simplified solid-state fermentation for preparing flour from non-decorticated cowpeas results in elimination of sucrose, stachyose and raffinose. Raffinose and stachyose, are hydrolyzed by intestinal anaerobic microorganisms to produce flatulence or intestinal gas (Calloway et al., 1971). Heating cowpeas sharply decreases folacin, niacin and riboflavin levels, but losses are recovered during fermentation (Prinyawiwatkul et al., 1996b). Zamora and Fields (1979) employed natural fermentation as a tool to upgrade the nutritional quality of cowpea. Similar fermented cowpea flour production was also reported by Lu and Sanni- Osomo (1988).

An increase in color saturation of fermented flours is said to be due to mainly to increased *b* (yellowness) values. A more intense color is also characteristic of paste made from flour milled from fermented cowpeas (Prinyawiwatkul et al., 1996b).

It was reported that methionine and isoleucine contents were markedly increased in fermented cowpea flours compared with those of nonfermented flours. Thiamine contents of nonfermented and fermented flours were the same, while niacin decreased and riboflavin increased in fermented flour. One advantage of fermentation over supplementation or fortification is that trypsin inhibitors, phytic acid, raffinose and stachyose can be decreased or eliminated (Zamora and Fields,

1979). On the other hand, an increase in tannin contents in fermented dry beans was reported (Ibrahim et al., 2002).

The responsible microorganisms in naturally fermented cowpeas are said to be *Lactobacillus casei*, *L. leichmanii*, *L. plantarum*, *Pediococcus pentasaceus*, and *P. acidilactici*. Although fermentation may be achieved by allowing a lengthy succession of naturally occurring microorganisms, the use of pure starter culture under aseptic conditions is more widely used in cottage and industrial-scale fermentations (Prinyawiwatkul et al., 1996b).

Prinyawiwatkul et al. (1993) prepared fermented, partially defatted peanut flour using solid-substrate fermentation with pure starter culture (*Rhizopus oligosporus*). For fermented cowpea flour production, cowpeas were washed in running water, dried at 50-55°C, ground through a 1-mm mesh screen, made a slurry by adding four times water and 5% sugar by weight of the flour, fermented at 25°C for 4 days, dried at 50-55°C for 48 hours and ground through a 1-mm mesh screen. They found that the presence of seed coats did not adversely affect fermentation and thus a decortication step was not necessary. The yield and loss of fermented cowpea flour was affected considerably by the fermentation process. The yield of fermented flour (about 74%) was significantly lower than that of the nonfermented control flour (96%). Solid loss occurred in soaking, boiling, fermentation and grinding steps. Fermented cowpea flour may be anticipated to have modified functional, nutritional, and sensory properties in food products in which it is incorporated.

1.5.4. Soaking and cooking

Soaking the beans reduces the antinutritional components. In a study, 16 h soaking was reported to remove 16.02% trypsin inhibitor by water and 16 h soaking with the addition of sodium bicarbonate removed 24.22% trypsin inhibitor by water. By soaking for the same period of time, there was also a considerable reduction in phytic acid and stachyose and complete removal of raffinose (Ibrahim et al., 2002).

Cooking may be done on beans with or without previous soaking in water, which typically reduces cooking time. Cooking beans in water, with or without pressure, increases the protein quality, and protein and carbohydrate digestibility, and it

inactivates protease and amylase inhibitors, as well as lectins (Bressani, 1993). This process also reduces the concentration of other antinutritional factors such as tannin and phytic acid (El Tabey Shehata, 1992). Elimination and redistribution of tannin in the cooked bean and cooking water contribute to an increased nutritive value. On the other hand, cooking increases dietary fiber content from around 19 to 26%, trapping protein and probably making it unavailable. Cooking induces loss of vitamins (25-30%) and minerals (10-15%) (Augustin et al., 1981). Dehulling followed by cooking increases protein quality and digestibility (Bressani, 1993). This effect is due to the removal of not only crude fiber but also of tannins, both of which are present in the hulls of the bean. Heat processing when done under controlled time and temperature, usually improves the protein quality of food grain legumes (Bressani, 1985).

In a study, with respect to oligosaccharides, cooking decreased stachyose content by 46% and raffinose by 50% (Onigbinde and Akinyele, 1983) probably due to leaching into water (Wang et al., 1997). The levels of reduction in tannins, phytic acid and trypsin inhibitor were about 40, 6.8-11.9% and 86.98-90.46% respectively by cooking where pressure cooking was more effective than ordinary cooking (Ibrahim et al., 2002).

1.6. Uses of Cowpea Flour in Foods

Cowpea flour, like soybean and peanut flours, contains a high amount of protein, thus representing a source of nutrition and potential functional ingredient in a wide range of products such as bread, macaronies, weaning foods, chips, cookies, biscuits, extruded snacks, etc. The use of cowpea flour to partially replace wheat flour in food products would have additional economic advantages for developing countries that depend on imported wheat (Okaka and Potter 1977). Bakery products supplemented with cowpea flours also provide a means for improving the nutritional quality of wheat flour based foods without considerably sacrificing palatability.

In bread making, the use of cowpea flours is limited due to decreased loaf volume. Okaka and Potter (1977) used drum dried cowpea flour made according to Onayemi and Potter (1976) for supplementing bread and they observed that progressive substantial decrease in loaf volume occurred as the cowpea flour content exceeded 10%. Addition of surfactants (sodium or calcium stearoyl-2-lactylate) minimized the

effect but 30% cowpea flour added breads were unacceptable even with the addition of surfactant.

Mustafa et al. (1986) added nondecorticated cowpea flour to yeast bread and observed that replacement of 10% wheat flour with cowpea flour produced acceptable bread with increased loaf volume; but more than 10% cowpea flour replacement decreased loaf volume and imparted a distinct beany flavor. Sales (1980) produced bread with cowpea flour obtained by simple dry-roast/dry-mill process at a ratio of 35:65 (cowpea:wheat flour, w/w).

Substituted bread with fermented and nonfermented cowpea flour increased the protein content from 10.8% to 14.2% and 13.2% respectively. Loaf volumes did not show an appreciable difference but the color of breads containing cowpea flour was slightly darker than the control. Fermented cowpea containing bread was more acceptable than nonfermented cowpea containing bread (Lu and Sanni-Osomo, 1988).

Sugar cookies (McWatters, 1978) and biscuits (McWatters, 1980) were substituted by 10, 20 and 30% nondecorticated cream type cowpea flour and the dough handling properties and baking characteristics were not significantly different from the control. A beany flavor was not distinct up to 30% cowpea flour addition.

Buttermilk doughnuts containing 10%, 20% and 30% cowpea meal of nondecorticated cream type cowpeas had sensory qualities that compared favorably with those of 100% wheat flour doughnuts. Cowpea meal including batters were well suited to mechanical cutting, dispensing and frying, however they absorb excessive oil during frying. This could be minimized by using finely milled flour rather than meal. The addition of fat controlling ingredients such as soy flour to the doughnut formula also improved the overall quality (McWatters, 1982).

Tortillas which are unleavened bread prepared from corn or wheat flour are staple food in many country. The usual ingredients are flour, salt, shortening and water with the flattened pieces of dough cooked in a pan or on a griddle. Substitution of wheat

flour with 0-24% of cowpea flour, resulted in tortillas with quality characters similar to 100% wheat flour (Holt, 1990).

Muffins are chemically leavened quick breads typically prepared with soft wheat flour. Holt et al. (1992) prepared muffins successfully with 43% cowpea flour addition.

Acceptable chips having lower fat (18-20%), and higher protein (6.5-7%) than commercial potato (43% fat and 5% protein) and corn (33% fat and 1.75% protein) chips were produced by 50% nonfermented and fermented cowpea flour addition (Lu and Sanni-Osomo, 1988).

Because of its availability and popularity in some regions of the world with hot climate, cereals and legumes are the main sources of nutrients for weaning children. So, baby food including cowpea flour with reduced beany flavor was prepared by Okaka and Potter (1979). These are not designed to replace breast milk for neonatal infants, but rather the transition to solid food necessitated by the increasing nutritional requirements of 6 months to 1.5 year-old children. Baby food with reduced beany flavored cowpeas was more favorable than the one with untreated cowpeas. The storage stability of the food was excellent for at least 16 weeks at 30°C. Because the production of most weaning and infant foods involves complicated technologies such as drum drying and extrusion cooking, a weaning food with high nutritional quality, low moisture content and ability to form a smooth paste after hydration with tapwater was prepared by using decorticated and pressure-dried pearl millet (70%) and cowpea (30%) (Almeida-Dominguez et al., 1993). The equipment used was simple that is suitable for low-income families in developing countries.

Chinese type noodles containing 4-12% cowpea flour and 7-21% defatted peanut flour were prepared by Chompreeda et al. (1988). The protein content of the product was increased to 21% by fortification of 15% cowpea with acceptable sensory quality.

Extruded cowpea flour alone and/or in combination with other legumes have been investigated with respect to changes in protein functional and nutritional qualities, available lysine, and total and reducing sugars. Because of its high protein content it is difficult to puff pure cowpea flour by extrusion. It is said that extruded products containing cowpea may be made more convenient, more stable, and more nutritious than traditional products consumed (Prinyawiwatkul et al., 1996a).

Akara is made from whipped cowpea paste flavored with chopped hot or bell pepper, onion and salt, dropped by tablespoon portions into hot oil and deep fried (McWatters et al., 1992). In especially West African countries, akara is the most common breakfast and snack food which contributes to the diet significantly. Besides home preparation, akara is sold in marketplace, too.

Whole seed cowpea meal is used to make imitation milk and yoghurt-like products (Schaffner and Beuchat, 1986). The greater fat binding capacity of protein from whole seed or dehulled seed flour compared with soy flour (Sosulski et al., 1987), makes cowpea a potential ingredient as an extender in meat formulations. The sensory quality of ground beef patties supplemented with 5% cowpea flour compared favorably with controls (McWatters, 1977).

Cowpea flour can be used as a supplement to pasta products, too. It was demonstrated that protein quantity is a key factor in obtaining good quality products (Novaro et al., 1993) because they promote the building up of a diffused and coagulated protein network capable of entrapping starch granules and preventing them from excluding from pasta during cooking (Resmini and Pagani, 1983). It was suggested that, without any surfactant and vital gluten addition, legume flours can be used in a limited amounts in pasta products (Markoni et al., 2000).

Bergman et al. (1994) were developed a high-temperature dried soft wheat pasta supplemented with 10, 20 and 30% cowpea meal. Maximum ash content was 1.3% with improved pasta color scores and integrity during cooking. According to the sensory data, no difference was found between samples. It was stated that, high temperature drying and cowpea flour addition could overcome some of the constraints of using soft wheat flour in pasta production.

For the aim of pasta enrichment, various types of protein sources were used. As an example processed peas were added with some emulsifiers to improve dough quality (Frias et al. 1997). Rice flour was used to supplement the pasta with the addition of emulsifiers, too (Lai, 2001). Marconi et al. (2000) produced functional pastas, enriched with β -glucans and dietary fiber. Although darker than durum wheat pasta, the pastas had good cooking qualities with regard to stickiness, bulkiness, firmness and total organic matter released in rinsing water. Wheat germ addition to pasta was applied on a level of 15%, with good sensory results (Pınarlı, Öner and İbanoğlu, 2004).

1.7. Spaghetti Production

Spaghetti is produced from the semolina of *Triticum durum* by proper mixing of semolina and water, and forming and, then drying. The type of wheat is very important in spaghetti production. Durum wheat makes it possible to obtain a strong and non-sticky dough for spaghetti production, better product color and superior cooking quality because of its strong gluten characteristics (Boyacıoğlu, 1992).

In spaghetti production, durum wheat is ground into semolina and used. There are a lot of advantages of using semolina instead of flour in spaghetti production. With semolina, less amount of water is needed to make dough, decreasing the time for drying and cost. Moreover semolina gives the product a better structure, it resists to cracking of the surface and keeps cooked spaghetti from dispersion (Tuncer and Ercan 1990, Boyacıoğlu and Ünal 1990).

The history of macaroni was said to be very old among the products made from wheat and it is thought to be produced in China as a noodle in the year of 5000 B.C. and to be brought to Italy that is supposed to be its motherland now by Marco Polo. Then, it spreaded into Europe and U.S.A. Macaroni that was previously homemade only industrialized firstly in Italy at the beginning of nineteenth century and progressively made with more modernized machines including hydrolic presses, mixers, driers, etc. through the twentieth century. In Turkey, the macaroni production started in 1922 as the first establishment of the macaroni sector (Akar, 1998).

During spaghetti production, the moisture content of semolina that has a particle size between 120-500 μ is adjusted to 30% by mixing with water for 15-30 minutes to obtain a homogenized dough under vacuum. The aim of vacuum application is to obtain a glassy, bright yellow coloured product without air pores over a surface of pasta. The shaping of the dough into various forms under vacuum is applied by a press machine and dried on a continuous belts or hangers including pre-drying, drying, resting and cooling steps (Akar, 1998).

Among the cereal products, macaroni with a long shelf-life, simple and easy manufacturing process, high nutritional value with respect to its price is one of the most popular food after bread (Özkaya, 1996). Turkey has an important place among the durum wheat producing and macaroni exporting countries. Annual consumption of macaroni in Turkey is 4-4.3 kg/person. This value is 4.5 kg/person in Germany, 8.5 kg/person in U.S.A., 27.0 kg/person in Italy (Anonymous, 1998).

There have been numerous studies on the improvement of nutritional quality of macaroni by adding protein-rich sources (Corta et al., 1990; Schmidt et al., 2003). Overall quality of durum wheat macaroni is influenced primarily by the properties of the protein and starch fraction. The functional properties of cowpea are suitable to be incorporated into protein-rich food products (Abbey and Ibeh, 1988).

1.8. *In Vitro* Protein Digestibility

The biological utilization of protein, in any food product, is primarily dependent on digestibility together with its amino acid profile. According to Rathi et al. (2004), the *in vitro* protein digestibility of pasta prepared from native pearl millet was relatively low and it was attributed to the presence of considerable amounts of some antinutrients in unprocessed or native pearl millet. It was stated that, the *in vitro* digestibility of legumes were increased during cooking and fermentation (Kiers et al., 2000).

1.9. Differential Scanning Calorimetry

Thermal stability is an important determinant of functional properties. This is because structural parameters that influence functionality are altered chemically

during the application of heat. Thermal treatment of varying severity is the most common process step during processing of foods. The change of the state of a substance is accompanied by a change in the energy level. Changes in the energy level can be manifested by the absorption of heat (endothermic reaction) and liberation of heat (exothermic reaction). The measuring principle in DSC is to compare the rate of heat flow to the sample of interest and to an inert material, which are heated or cooled at the same rate. Changes associated with absorption or evolution of heat in the sample cause a differential heat flow, which is recorded graphically as a thermogram. DSC has been employed to study effects of heat induced phase transitions of starch and protein systems (Henshaw et al., 2003).

Differential scanning calorimetry (DSC) is a thermo-analytical technique for monitoring changes in physical and chemical properties of materials as a function of temperature. More studies have been reported on thermal behaviour of starch and protein as isolated preparations than on these constituents' thermal behaviour as they occur in a food system (Henshaw et al., 2003) such as spaghetti which contains starch and protein as major components.

Several studies (Choi and Kerr, 2004; Hibi, 1998) have been done to help understand the molecular mechanism of the process and quantify the influence of sugar, salts, lipids and varying amounts of moisture content on the starch gelatinization phenomena.

1.10. Antioxidants

Many types of antioxidant molecules including ascorbic acid, tocopherols, carotenoids and many phenolic substances exist naturally in foods in different amounts. The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body (Namiki, 1990). The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases (Papas, 1999).

Different antioxidants can be measured in laboratories separately, but the measurements are not practical and antioxidant effects of different antioxidant molecules are additive, the total antioxidant capacity (TAC) of a sample is measured. Various methods have been developed to measure TAC. In general, a radical is generated in the assay, and the antioxidant response of the sample against the radical is measured. The most widely used methods for TAC measurement are colorimetric methods. Trolox (the water soluble analogue of vitamin E) as the most widely used traditional standard has been used for the calibration of the assays (Erel, 2004a). Some processes affect TAC of foods. TAC values of processed cowpea samples and spaghetti samples with and without these cowpea flours were evaluated. It was aimed to understand how fermentation, germination, cooking processes affected TAC of cowpea flour samples and spaghetti samples including them.

Total antioxidant capacity measurements can be used to estimate the antioxidant status of samples measured (Miller et al., 1993). It is known that processing may affect the antioxidant capacity of foods negatively or positively, depending on the type and nature of the process being used.

1.11. The Objective of The Thesis

The purpose of this thesis is to analyse the effect of fermentation, germination and cooking processes on the selected properties of cowpea flour. It was also aimed to incorporate the processed and unprocessed cowpea flour into standard spaghetti formulation to increase its nutritional value and obtain an acceptable product that is suitable for consumption.

2. MATERIAS AND METHODS

2.1. Cowpea Flour Production

Dry black eyed type cowpeas were purchased from a local market in Gaziantep, Turkey. The seeds were handsorted to remove wrinkled, moldy seeds and foreign material.

Control (unprocessed) cowpea flour: Cowpeas were soaked in distilled water for 10 hours at room temperature, decorticated manually, dried at 60°C for 8 hours in an oven (FTR3400E, Profilo, Turkey) and milled to flour by a lab scale hammer mill (Brook Crompton, Series 2000, England) to pass a 20 mesh screen.

Germinated cowpea flour: Cowpeas were soaked in distilled water for 10 hours at room temperature. The hydrated seeds were spread between clean moist cotton sheets over a metal tray. Germination took place at 30°C for 24, 48, 72 hours in a thermostatically controlled oven (Nüve, EN500, Turkey). Germinated cowpeas' roots and testa were rubbed off by hand and seeds were dried at 60°C for 8 hours in an oven (FTR3400E, Profilo, Turkey) followed by milling to flour by a lab scale hammer mill (Brook Crompton, Series 2000, England) to pass a 20 mesh screen.

Fermented cowpea flour: Control (unprocessed) cowpea flour was mixed with distilled water (1:4, wt/wt) to form a slurry which was left for fermentation at 30°C for 24, 48, 72 hours in a thermostatically controlled oven (Nüve, EN500, Turkey) by its indigeneous flora. The surface of the slurry was covered by aluminium foil to prevent dehydration. Fermented cowpea slurries were dried at 60°C for 8 hours (FTR3400E, Profilo, Turkey) and milled to flour by a lab scale hammer mill (Brook Crompton, Series 2000, England) to pass a 20 mesh screen.

Cooked cowpea flour: Cowpeas were soaked in distilled water for 10 hours at room temperature, decorticated manually, cooked in boiling excess water for 10 minutes

dried at 60°C for 12 hours in an oven (FTR3400E, Profilo, Turkey) and milled to flour by a lab scale hammer mill (Brook Crompton, series 2000, England) to pass a 20 mesh screen.

2.2. Spaghetti Production

Spaghetti samples were prepared using a pilot scale pasta-making device consisting of a mixer-press (Namad-Microimpianti Lab. Cereal C. Garampi, Italy) and a drier (L. Giussoni Tavola Psicrometrica fara diadoda, Bergamo, Italy). The processed cowpea flours i.e. control, germinated, fermented and cooked were added to durum wheat semolina at 10, 15, 20 and 25 % (w/w) levels based on the semolina used (Table 2.1).

Table 2.1. Blends of semolina and cowpea flours used in spaghetti production

Designation	Blends
10U	10% unprocessed cowpea flour, 90% semolina
10G	10% germinated cowpea flour, 90% semolina
10F	10% fermented cowpea flour, 90% semolina
10C	10% cooked cowpea flour, 90% semolina
15U	15% unprocessed cowpea flour, 85% semolina
15G	15% germinated cowpea flour, 85% semolina
15F	15% fermented cowpea flour, 85% semolina
15C	15% cooked cowpea flour, 85% semolina
20U	20% unprocessed cowpea flour, 80% semolina
20G	20% germinated cowpea flour, 80% semolina
20F	20% fermented cowpea flour, 80% semolina
20C	20% cooked cowpea flour, 80% semolina
25U	25% unprocessed cowpea flour, 75% semolina
25F	25% fermented cowpea flour, 75% semolina
25C	25% cooked cowpea flour, 75% semolina
S (Control)	100% semolina

Each blend sample was mixed with water that the final moisture content of the dough was 33 % wb. The mixing operation was applied for 15 minutes. Then the dough was put into the extruder and the upper side of the equipment was closed. The pressure was arranged to 600 mm Hg and the machine was run with a die of multiple opening. After the extrusion, the drying took place in a ventilated static system for 20 hours at 40°C and 60 % relative humidity in the dryer. In the last hour the heater was switched off and only the ventilator was run in the dryer. The final moisture content of the product after drying was 10-12 %. The thickness of the pasta samples were approximately 1.7 mm after dryer. The samples were kept in polyethylene bags (LDPE, water vapor permeability: 2.5g/m² 50µm; oxygen permeability: 4000 cm³/m²bar 200µm) and stored in the dark at room conditions until used. The spagetties were left undisturbed for 10 days according to D'Egidio *et al.* (1982).

2.3. Chemical Analysis

2.3.1. Determination of ash and moisture content

Total ash and moisture contents of cowpea flours and spaghetti samples were determined by using the standard methods (AACC, 1990). Tests were done in triplicate and results were expressed on dry basis. For moisture content determination, approximately 5 grams of sample were weighed into glass petris and put into the oven in 130°C until constant weight and then weighed again. For ash determination about 2 grams of sample were heated in a muffle furnace at 900°C until constant weight. The results were calculated in dry basis.

2.3.2. Determination of protein content

Cowpea flours and spaghetti samples were analysed for their crude protein by using the Kjeldahl method (AACC, 1990) with a conversion factor of 6.25 and 5.70 for cowpea flour and semolina, respectively. All tests were done in triplicate and results were expressed on dry basis. Chemicals used were analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO). For this purpose, approximately 2 grams of pounded sample, 10 grams of K₂SO₄ and a little amount of Cu₂SO₄ with 25 ml of concentrated H₂SO₄ were taken into digestion tubes with boiling cheeps and digested at 450°C for 40 min until green color formation was observed. Then it was cooled to room temperature and put in the distillation chamber. In the distillation equipment, to neutralize the sulphuric acid, both NaOH (concentration: 50%) and water were added

in 5ml amounts. The ammonium formed was distilled into 25 ml H₃BO₄ solution (4% g/l). The indicator of methylene red was used and the solution was titrated with 0.1 N HCl to the pink colour. The amount of HCl used was proportional to amount of nitrogen present in the sample. The factors used to convert nitrogen content to the protein values were 5.70 for wheat semolina and 6.25 for cowpea flour (AACC, 1990). Then the values were calculated in dry basis.

2.3.3. Determination of crude oil content

Cowpea flours and spaghetti samples were analysed in a laboratory scale solvent extraction equipment (Ser 148 extraction unit by solvent; Velp Scientifica). About 8 grams of samples were taken into cartridges and extracted with hexane followed by immersion, washing and recovery steps (AACC, 1990). The results were calculated in dry basis. All tests were done in triplicate.

2.3.4. Determination of total titratable acidity

During fermented cowpea flour production, acidity of the flour samples were analysed by titration method. A 100 ml solution was prepared by weighing 10 grams of flour sample and 90 ml of distilled water and and this solution was titrated with 0.01 N NaOH after addition of phenolphthalein as an indicator. Acidity was calculated as a lactic acid (LA%) (AACC, 1990).

2.3.5. Determination of carbohydrate

Carbohydrate amount of cowpea flours and spaghetties were calculated by difference by summing up the protein, ash, crude oil contents of them and subtracting the value from 100.

2.4. Color

Color of dried spaghetti samples was evaluated by measuring the *L* (100=white; 0=black), *a* (+, red; -, green) and *b* (+, yellow; -, blue) values by means of a Hunter Lab Color QUEST II (Virginia, ABD) with illuminant D₆₅ as reference. The values were the mean of three determinations. The equipment was calibrated using a white (C6299) and a gray (C6299G) standards.

2.5. Sensory Analysis of Textural Attributes

A 100 gr dry spaghetti sample (wb) was cooked in one liter tap water for 13 minutes and drained for 10 sec to remove excess water, and then placed into a dish. After 9 minutes of draining, spaghetties were analysed for their stickiness, firmness, bulkiness and total organic matter (TOM).

Textural assessment was made by a trained panel of three experts (assessors) (D'Egidio et al., 1982). The stickiness, bulkiness and firmness of the cooked spaghetti samples were determined according to the procedure reported by Cubadda (1988). The scale for the stickiness, firmness and bulkiness was given in Table 2.2.

Table 2.2. Scale for sensory analysis of textural attributes of spaghetti

Criteria	Degree	Point
Stickiness	very high	0-15
	high	16-30
	medium	31-40
	little	41-55
	too little	56-75
	absent	76-100
Firmness	very soft	0-15
	little resistance to crushing	16-30
	medium resistant to crushing	31-40
	acceptable	41-55
	good	56-75
	very good	76-100
Bulkiness	very high	0-15
	high	16-30
	medium	31-40
	acceptable	41-55
	too little	56-75
	absent	76-100

The degree of stickiness, which is the amount of material that adheres to the surface of the cooked pasta was assessed visually (with the aid of standard reference samples) and by handling the samples. Bulkiness, which was assessed in the same way, is the degree to which the strands of pasta adhere to each other. Firmness is the degree of resistance of the cooked pasta when either pressed between the fingers or chewed. These parameters were rated on a six-point hedonic scale and then converted into numerical scores.

The total score regarding cooking quality was obtained from arithmetic mean of the scores given by the panelists. The final score was correlated with a description that spaghetti with a total score of 40 was a poor or mediocre quality; >40 to 50 was not completely satisfactory; >50 to 70 was fair; >70 to 80 was good; and >80 was excellent.

2.6. Total Organic Matter (TOM)

The amount of surface material released in the washing water after thoroughly rinsing the cooked spaghetti was assessed as total organic matter (TOM) and determined using the standard chemical method (Method 153, ICC 1995; D'Egidio et al., 1982). For this purpose, after the evaluation of stickiness, firmness and bulkiness values were appraised, the spaghetti was poured into 500 ml water at room temperature for 12 min after the 9 minutes of rest after cooking. During this time it was stirred three times at every 4 minutes. From the well stirred washing water suspension, 5.0 ml of washing water was pipetted into a 600 ml beaker and then evaporated at 80°C in an oven. After complete evaporation, further heating was avoided. 10.0 ml of 1N $K_2Cr_2O_7$ was added from a burette, wetting the residue completely; then 20 ml of a 96% solution of H_2SO_4 was pipetted under a hood. This was mixed for 1 min and allowed to react for 30 min. The mixture was diluted with 200 ml of H_2O , and excess $K_2Cr_2O_7$ was titrated with 0.5 N $Fe(NH_4)_2(SO_4)_2$. Diphenylamine (0.5%) in concentrated H_2SO_4 was used as an indicator. The end of the titration was indicated by a change of color from violet to green. Results were expressed as grams of starch obtained from 100 grams of spaghetti.

For the practical evaluation of spaghetti products, the following classification values were (D'Egidio and Nardi, 1996): very good quality: pasta or spaghetti with TOM

values lower than 1.4; good quality: pasta or spaghetti with TOM values between 1.4 and 2.1; low quality: pasta or spaghetti with TOM values higher than 2.1.

2.7. Phytic Acid Determination

Phytic acid was measured by a colorimetric method according to Haug and Lantzsch (1983). Phytic acid in the sample (0.3 g) was extracted with the solution of HCl (0.2 N, 50 ml) and 0.5 ml of this solution was precipitated with 1 ml solution of Fe III (ammonium iron (III) sulphate.12 H₂O) (0.01 g Fe / ml). Fe amount in the serum part was measured with a spectrophotometer at 519 nm (Haug and Lantzsch, 1983) against distilled water and phytic acid amount was calculated from this value.

2.8. Total Antioxidant Capacity (TAC) Determination

Total antioxidant capacity measurement was done by using a novel, colorimetric method of Erel (2004b) where ortho-dianisidine radicals were used as an oxidizing agent. 0.5 g of the sample was taken for each analysis. The results were given in $\mu\text{mol Trolox equiv/gr}$ as measured with spectrophotometer at 660 nm. (Erel, 2004b). All chemicals were ultra pure grade and purchased from Sigma Co. and Merck Co.

2.9. Microbial Analysis of Spaghetti Samples

A 25.0 g spaghetti sample was weighed aseptically into a sterile glass flask (500 ml) and mixed with 225 ml of a sterile peptone-water solution (0.1% peptone) using a magnetic stirrer for 20 minutes to obtain a homogenized sample (Jay, 1986).

2.9.1. Aerobic plate count (APC)

The aerobic plate count was made using the aerobic spread plate count method described by Jay (1986). Aerobic plate count agar (PCA; Merck, Darmstadt, Germany) was used for the analysis.

Dilutions of the sample were prepared from 1 to 1.10^{-7} and an amount of 0.2 ml from every dilution was transferred onto a corresponding labeled petri dish and spread-plated over the agar surface. Inoculated PCA plates were incubated at 35°C for 24-72 hours. The plates with less than 300 colony forming units (CFU) were counted and the average value was taken after a duplicate count. The number of CFUs were multiplied by the dilution factor and divided by the inoculation amount in order to

determine the CFUs per gram of spaghetti. The CFU numbers were transformed into corresponding logarithmic numbers.

2.9.2. Mold and yeast counts

Mold and yeast counts were made with the aerobic spread plate count method described by Jay (1986). Potato dextrose agar (PDA; Merck, Darmstadt, Germany) was used for the analysis.

A 0.2 ml of the dilution prepared from 1 to 1.10^{-7} was transferred onto a corresponding labelled plate and spread-plated over the agar surface. Inoculated PDA plates were incubated at 25°C for 2-5 days. The plates with less than 300 colony forming units (CFU) were counted and the average value was taken after a duplicate count. The number of CFUs were multiplied by the dilution factor and divided by the inoculation amount in order to determine the CFUs per gram of spaghetti. The CFU numbers were transformed into corresponding logarithmic numbers.

2.10. *In Vitro* Protein Digestibility

Uncooked spaghetti samples were investigated for their *in vitro* protein digestibilities by the modified methods of Hsu et al. (1977) and Dahlin and Lorenz (1993). The samples were ground to fine powder that was able to pass through 80 mesh screen. The solutions were prepared by using distilled water. Fifty milliliters of aqueous protein suspension based on crude protein content (6.25 mg protein/ml) were allowed to rehydrate for 60 minutes at 5°C with intermittent mixing. After rehydration, samples were placed in a 37°C water bath and the pH was adjusted to 8.00 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 was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl. Five ml of enzyme solution were 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 15 s after enzyme addition and at one minute intervals for ten minutes. Triplicate analysis was performed for each sample. The enzyme solution was freshly prepared before each series of the test.

The percent protein digestibility (Y) was calculated by the following equation (Hsu et al., 1977):

$$Y=210.464-18.1X$$

where X is the change in pH after 10 minutes ($\text{pH}_{t=10}-\text{pH}_{t=0}$).

2.11. Differential Scanning Calorimetry

A Perkin-Elmer DSC 6 (Perkin Elmer Ltd, Norwalk, CT) instrument was used to analyse dry spaghetti samples. The instrument was calibrated with indium, and the instrument sensitivity was 0.5 mcal/s. Ground spaghetti samples made of 20% fermented, germinated, cooked and unprocessed cowpea flour and a sample made of durum wheat without cowpea flour were sieved through a 425 μm mesh screen. Samples of known moisture content were carefully wetted with distilled water with micropipette to give a paste with a ratio of dry sample to water of 1:3 (Güler et al., 2002; Villwock et al. 1999). Samples were weighed into aluminum standart differential scanning calorimetry (DSC) pans with micropipette, sealed and allowed to equilibrate for 1.5 h prior to analysis (Pınarlı, Öner and İbanoğlu, 2004). A sample size of 12 \pm 3 mg was used. The samples were heated at a rate of 5°C/min from 30 to 130°C with nitrogen flushing rate of 40cm³/min. A sealed empty pan was used as the reference. The starch gelatinization characteristics in the DSC thermogram can be explained by various defined temperatures. By using instrument's analysis software programme, for each endotherm, the onset temperature (T_o), the peak temperature (T_p) and entalpy (ΔH , the amount of energy required for gelatinization) were computed, peak height index (PHI) was calculated as the ratio of $\Delta H/(T_p-T_o)$ (Krueger et al., 1987). Entalpies were reported on a dry sample basis.

2.12. Statistical Analyses

All determinations were done triplicate and mean \pm standart deviation (SD) values were calculated. A commercial software programme (SPSS 11.0, SPSS Inc. 2001) was used to perform statistical analyses. Data were assessed by analysis of variance (ANOVA). Duncan's multiple range test was used to seperate means. Significance was $\alpha=0.05$ throughout the analysis.

3. RESULTS AND DISCUSSION

3.1. Cowpea Flour Production

It has been stated that legumes should be processed before using in the food formulations, due to the presence of antinutritional factors. Due to unacceptable sensory evaluation scores, there exist some restrictions on cowpea flour addition in higher concentrations even if the antinutritional factors were decreased by some processes like soaking, germination, fermentation, cooking, pressure cooking (Mustafa et al., 1986). Germination, fermentation and soaking have been applied to various legumes to totally/partially eliminate antinutritional factors such as phytic acid and trypsin inhibitor (Wang et al., 1997).

During the trial period of the fermentation process, acidity data was taken, and increased sharply during the first day from 0.22% to 0.52% and in the following days it increased more slowly (Fig. 3.1).

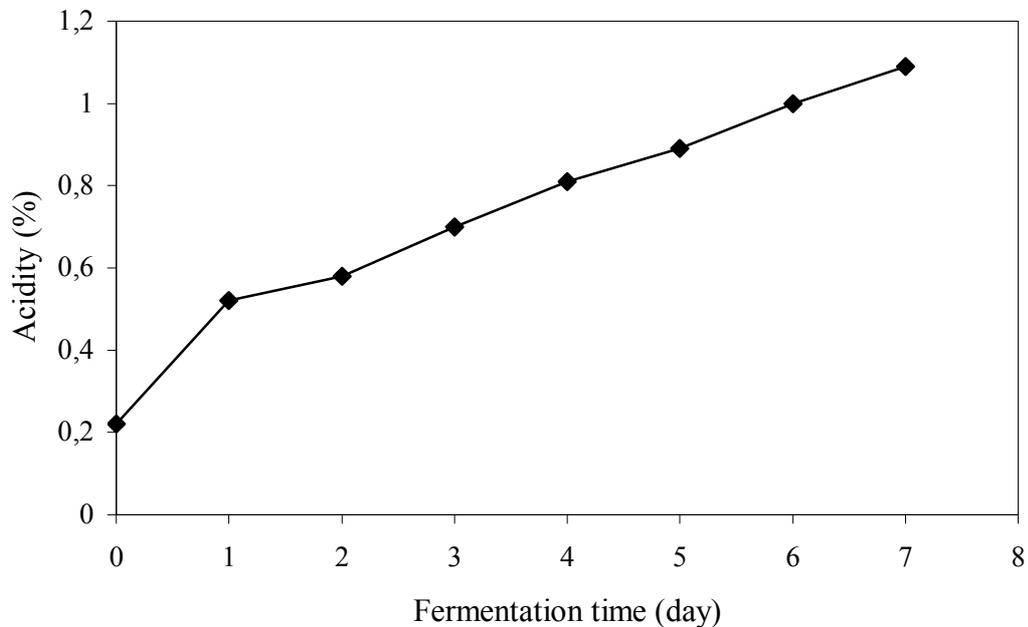


Figure 3.1. Acidity of cowpea flours as lactic acid (%), (dry weight basis)

During the fermentation of cowpeas, the acidity increased rapidly from 0.22 to 0.52 % in 24 hours at 30°C, although the time for adequate fermentation was found as 48 hours in a different study (Lu and Sanni-Osomo, 1988). But in that study cowpeas were fermented as grain seeds rather than flour which could extend the time. In another study, the pH of cowpea flour decreased from 7.04 to 6.18, 4.60 and 4.52 at the end of 5.8, 20 and 34 hours at 30°C respectively. Experimental acidity values after 5.8, 20 and 34 hours of fermentation were 0.43, 0.88 and 1.10 % as lactic acid respectively (İbanoğlu and İbanoğlu, 2001).

In this study, germination and fermentation were applied for 24 hours as suggested by Nnanna et al. (1990). It was reported that, from a nutritional point of view, prolonged processes should be avoided because they may lead to significant total solids and nutrient losses and unnecessary expenditures of energy and time (Prinyawiwatkul et al., 1996b).

The presence of fermentable carbohydrates in cowpea flour eliminated the need of carbohydrate addition and the cowpea flour was fermented by its indigenous microflora. Fermentation was applied to cowpea flour instead of cowpea legume to facilitate the fermentation process to upgrade the nutritional quality of cowpea flour as applied by Zamora and Fields (1979) and Lu and Sanni-Osomo (1988).

It has been also stated that soaking is an important step in processing legumes and therefore not to be eliminated from the whole process (Prinyawiwatkul et al., 1996b). In this study soaking was applied as 10 hours at room temperature before all types of processes applied on cowpeas to produce flour. Also during the production of unprocessed, fermented, germinated and cooked cowpea, decortication was applied because in the previous studies decortication was said to lead to a faster cooking time, increased digestibility, and better texture and appearance (Hudda, 1983) although it may not be necessary for production of steamed and fried cowpea products, provided that cowpea cultivars with a light-colored seed coat and little pigmentation in the hilum are employed (Prinyawiwatkul et al., 1994).

For the cooking process, when the combined effect of soaking and cooking on the nutrients with loss of them and antinutritional factors were taken into account from

the results of the previous studies (Wang et al., 1997; Ibrahim et al., 2002; Bressani, 1993; Onigbinde and Akinyele, 1983; Augustin et al., 1981) the time was chosen as 10 minutes. All types of cowpea flours were stored at room temperature (~25°C) until analysed during the experiments.

3.2. Spaghetti Production

In this study, 16 different spaghetti samples were produced (Table 2.1) with the exception of 25% germinated cowpea flour added spaghetti sample that could not be produced because of some technical limitations.

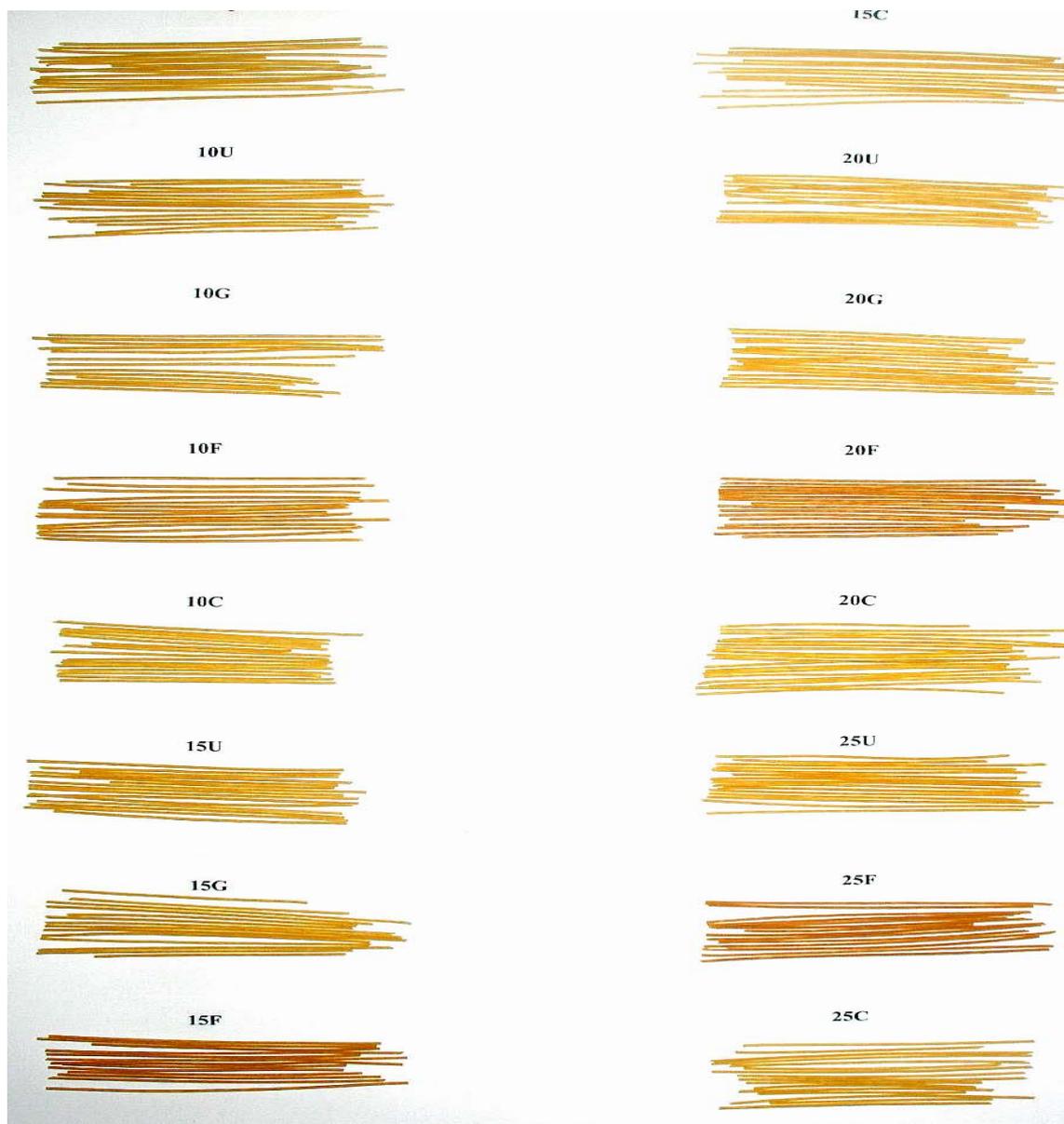


Figure 3.2. Spaghetti samples
See Table 2.1, for abbreviations

3.3. Chemical Analysis

The protein, crude oil, ash and carbohydrate content of the cowpea flours used are given in Table 3.1. Though, one day fermented and one day germinated cowpea flours were used in the spaghetti production, chemical analysis of these flours were done up to three days of the processes to observe the effect of processing time on the composition of the flours. Protein contents of the cooked and one day germinated cowpea flours decreased significantly ($p>0.05$) as compared to unprocessed flour.

Table 3.1. Composition of cowpea flours and semolina used ^a (\pm SD, %, dry weight basis)

Sample	Moisture	Protein	Crude oil	Ash	CHO ^b
Unprocessed	6.8 \pm 0.01 (e)	24.1 \pm 0.53 (ab)	2.1 \pm 0.03 (a)	3.3 \pm 0.03 (a)	70.5 \pm 0.20 (e)
Germinated(1) ^c	7.8 \pm 0.07 (d)	23.3 \pm 0.06 (cd)	2.0 \pm 0.01 (ab)	3.4 \pm 0.04 (a)	71.3 \pm 0.06 (c)
Germinated(2) ^c	8.2 \pm 0.25 (cd)	23.5 \pm 0.13 (bc)	2.0 \pm 0.01 (ab)	3.5 \pm 0.11 (a)	71.0 \pm 0.13 (cd)
Germinated(3) ^c	8.4 \pm 0.13 (bc)	24.7 \pm 0.26 (a)	2.0 \pm 0.06 (b)	3.4 \pm 0.01 (a)	69.9 \pm 0.11 (f)
Fermented(1) ^c	5.1 \pm 0.49 (f)	24.0 \pm 0.06 (b)	2.0 \pm 0.01 (ab)	3.3 \pm 0.01 (a)	70.7 \pm 0.04 (e)
Fermented(2) ^c	5.6 \pm 0.46 (f)	23.8 \pm 0.26 (bc)	2.0 \pm 0.06 (ab)	3.3 \pm 0.04 (a)	70.9 \pm 0.04 (d)
Fermented(3) ^c	6.3 \pm 0.22 (e)	24.1 \pm 0.01 (ab)	2.0 \pm 0.04 (ab)	3.3 \pm 0.01 (a)	70.6 \pm 0.08 (e)
Cooked	9.0 \pm 0.17 (ab)	22.8 \pm 0.35 (d)	2.0 \pm 0.03 (ab)	2.8 \pm 0.04 (b)	72.4 \pm 0.13(b)
Semolina	9.5 \pm 0.04 (a)	11.3 \pm 0.07 (e)	1.4 \pm 0.03 (c)	1.0 \pm 0.33 (c)	86.3 \pm 0.11 (a)

^a Figures in the same column sharing a common letter in the paranthesis are not significantly different at 0.05 level.

^b Carbohydrate by difference

^c Numbers in the paranthesis shows the number of the days of the process

While germination and fermentation may cause the loss of nitrogen containing compounds during processing, cooking may cause complex formation of nitrogen containing compounds with other compounds present in the cowpea flour, giving a reduced protein content as measured by Kjeldahl method (Ibrahim et al., 2002). The crude oil content of the cowpea flours remained unchanged ($p>0.05$) for all flours studied. It was reported that crude oil content of cowpea flours decreased slightly during fermentation due to the presence of some microorganisms hydrolysing triglycerides to yield free fatty acids (Beuchat, 1976). Ash content of cooked cowpea

flour decreased significantly ($p>0.05$) which might be due to the leaching of some minerals together with some soluble materials to the cooking water. Ash content of peanut flour has also been reported to increase upon fermentation (Quinn et al., 1975).

The results of moisture, ash, protein, crude oil and carbohydrate contents of the spaghetti samples are given in Table 3.2-9.

Table 3.2. Composition of unprocessed cowpea flour added spaghetti samples with the control sample^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
S (I)	7.71 \pm 0.13(f)	11.65 \pm 0.23(d)	1.34 \pm 0.03(e)	1.08 \pm 0.03(f)	85.93 \pm 0.10(a)
(6)	6.43 \pm 0.03(i)	11.80 \pm 0.28(d)	1.33 \pm 0.03(e)	1.08 \pm 0.08(f)	85.79 \pm 0.16(a)
(12)	7.98 \pm 0.04(e)	11.56 \pm 0.20(d)	1.32 \pm 0.01(e)	1.06 \pm 0.06(f)	86.06 \pm 0.20(a)
10U (I)	8.86 \pm 0.08(a)	12.99 \pm 0.58(bc)	1.41 \pm 0.04(cd)	1.27 \pm 0.01(e)	84.33 \pm 0.17(c)
(6)	7.13 \pm 0.10(g)	13.05 \pm 0.35(bc)	1.40 \pm 0.01(cd)	1.28 \pm 0.01(de)	84.27 \pm 0.11(c)
(12)	8.15 \pm 0.07(cde)	12.91 \pm 0.16(c)	1.37 \pm 0.01(de)	1.27 \pm 0.04(e)	84.45 \pm 0.14(c)
15U (I)	8.24 \pm 0.08(cd)	12.93 \pm 0.10(c)	1.43 \pm 0.03(bc)	1.44 \pm 0.06(bc)	84.20 \pm 0.07(c)
(6)	6.79 \pm 0.16(h)	13.40 \pm 0.21(bc)	1.42 \pm 0.02(cd)	1.40 \pm 0.06(cde)	83.78 \pm 0.04(b)
(12)	7.71 \pm 0.07(f)	13.09 \pm 0.16(bc)	1.40 \pm 0.03(cd)	1.36 \pm 0.06(cde)	84.15 \pm 0.21(c)
20U (I)	8.32 \pm 0.01(bc)	13.36 \pm 0.71(bc)	1.49 \pm 0.01(a)	1.48 \pm 0.04(bc)	83.67 \pm 0.20(d)
(6)	6.99 \pm 0.16(gh)	13.71 \pm 0.24(b)	1.48 \pm 0.01(ab)	1.41 \pm 0.10(cde)	83.40 \pm 0.17(d)
(12)	8.03 \pm 0.10(de)	13.60 \pm 0.14(bc)	1.48 \pm 0.03(ab)	1.42 \pm 0.04(cd)	83.50 \pm 0.14(d)
25U (I)	8.48 \pm 0.10(b)	14.69 \pm 0.27(a)	1.53 \pm 0.04(a)	1.67 \pm 0.04(a)	82.11 \pm 0.08(f)
(6)	6.14 \pm 0.11(j)	14.55 \pm 0.35(a)	1.50 \pm 0.01(a)	1.62 \pm 0.11(a)	82.33 \pm 0.03(ef)
(12)	7.71 \pm 0.13(f)	14.45 \pm 0.21(a)	1.50 \pm 0.01(a)	1.56 \pm 0.06(ab)	82.49 \pm 0.23(e)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.3. Composition of germinated cowpea flour added spaghetti samples with the control sample^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
S (I)	7.71 \pm 0.13(d)	11.65 \pm 0.23(c)	1.34 \pm 0.03(cd)	1.08 \pm 0.03(d)	85.93 \pm 0.10(a)
(6)	6.43 \pm 0.03(g)	11.80 \pm 0.28(c)	1.33 \pm 0.03(d)	1.08 \pm 0.08(d)	85.79 \pm 0.16(a)
(12)	7.98 \pm 0.04(c)	11.56 \pm 0.20(c)	1.32 \pm 0.01(d)	1.06 \pm 0.06(d)	86.06 \pm 0.20(a)
10G (I)	8.46 \pm 0.01(b)	13.05 \pm 0.29(b)	1.42 \pm 0.04(ab)	1.33 \pm 0.03(bc)	84.02 \pm 0.08(c)
(6)	7.12 \pm 0.13(e)	12.97 \pm 0.18(b)	1.40 \pm 0.02(abc)	1.28 \pm 0.04(bc)	84.35 \pm 0.07(b)
(12)	8.08 \pm 0.06(c)	12.93 \pm 0.18(b)	1.37 \pm 0.01(bcd)	1.25 \pm 0.04(c)	84.45 \pm 0.14(b)
15G (I)	9.20 \pm 0.14(a)	13.65 \pm 0.27(ab)	1.45 \pm 0.02(a)	1.41 \pm 0.03(b)	83.49 \pm 0.11(de)
(6)	7.04 \pm 0.23(ef)	13.44 \pm 0.25(ab)	1.44 \pm 0.02(a)	1.40 \pm 0.08(b)	83.72 \pm 0.06(cd)
(12)	7.72 \pm 0.04(d)	13.55 \pm 0.14(ab)	1.41 \pm 0.01(ab)	1.37 \pm 0.04(bc)	83.67 \pm 0.18(d)
20G (I)	8.48 \pm 0.04(b)	13.18 \pm 0.85(b)	1.45 \pm 0.07(a)	1.58 \pm 0.01(a)	83.79 \pm 0.22(cd)
(6)	6.83 \pm 0.17(f)	14.14 \pm 0.23(a)	1.44 \pm 0.01(a)	1.55 \pm 0.07(a)	82.87 \pm 0.06(f)
(12)	7.91 \pm 0.10(cd)	13.78 \pm 0.17(ab)	1.43 \pm 0.03(ab)	1.53 \pm 0.04(a)	83.26 \pm 0.20(e)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.4. Composition of fermented cowpea flour added spaghetti samples with the control sample^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
S (I)	7.71 \pm 0.13(cde)	11.65 \pm 0.23(e)	1.34 \pm 0.03(f)	1.08 \pm 0.03(g)	85.93 \pm 0.10(a)
(6)	6.43 \pm 0.03(fg)	11.80 \pm 0.28(e)	1.33 \pm 0.03(f)	1.08 \pm 0.08(g)	85.79 \pm 0.16(a)
(12)	7.98 \pm 0.04(bcd)	11.56 \pm 0.20(e)	1.32 \pm 0.01(f)	1.06 \pm 0.06(g)	86.06 \pm 0.20(a)
10F (I)	8.42 \pm 0.04(a)	12.91 \pm 0.13(d)	1.40 \pm 0.01(de)	1.42 \pm 0.04(de)	84.27 \pm 0.11(bc)
(6)	6.71 \pm 0.13(f)	12.95 \pm 0.23(d)	1.39 \pm 0.02(de)	1.23 \pm 0.08(f)	84.43 \pm 0.10(bc)
(12)	7.87 \pm 0.04(cde)	12.88 \pm 0.17(d)	1.38 \pm 0.01(e)	1.24 \pm 0.06(f)	84.50 \pm 0.14(b)
15F (I)	8.57 \pm 0.04(a)	12.95 \pm 0.07(d)	1.45 \pm 0.07(bc)	1.38 \pm 0.03(de)	84.22 \pm 0.04(bc)
(6)	7.80 \pm 0.14(cde)	13.27 \pm 0.17(d)	1.45 \pm 0.03(bc)	1.38 \pm 0.10(de)	83.90 \pm 0.03(c)
(12)	7.72 \pm 0.08(cde)	13.15 \pm 0.21(d)	1.42 \pm 0.01(cd)	1.34 \pm 0.08(ef)	84.09 \pm 0.23(bc)
20F (I)	8.24 \pm 0.06(ab)	14.33 \pm 0.28(bc)	1.47 \pm 0.02(ab)	1.52 \pm 0.04(bcd)	82.68 \pm 0.08(ef)
(6)	6.47 \pm 0.11(fg)	13.92 \pm 0.33(c)	1.47 \pm 0.03(ab)	1.50 \pm 0.04(cd)	83.11 \pm 0.37(de)

Table 3.4. continued

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
(12)	7.56±0.20(e)	13.90±0.14(c)	1.45±0.01(bc)	1.45±0.03(cde)	83.20±0.14(d)
25F (I)	8.05±0.07(bc)	14.80±0.28(ab)	1.47±0.05(ab)	1.67±0.06(a)	82.06±0.10(g)
(6)	6.33±0.10(g)	15.06±0.20(a)	1.49±0.04(a)	1.65±0.07(ab)	81.80±0.14(g)
(12)	7.62±0.11(de)	14.72±0.11(ab)	1.46±0.03(ab)	1.57±0.04(abc)	82.25±0.21(fg)

^a Figures in the same column sharing a common letter in the paranthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.5. Composition of cooked cowpea flour added spaghetti samples with the control sample^a (± SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
S (I)	7.71±0.13(bcd)	11.65±0.23(g)	1.34±0.03(b)	1.08±0.03(g)	85.93±0.10(a)
(6)	6.43±0.03(g)	11.80±0.28(g)	1.33±0.03(b)	1.08±0.08(g)	85.79±0.16(a)
(12)	7.98±0.04(b)	11.56±0.20(g)	1.32±0.01(b)	1.06±0.06(g)	86.06±0.20(a)
10C (I)	8.63±0.16(a)	12.86±0.26(f)	1.40±0.04(ab)	1.25±0.04(de)	84.49±0.10(b)
(6)	6.76±0.06(ef)	12.68±0.24(f)	1.39±0.01(ab)	1.21±0.04(ef)	84.72±0.11(b)
(12)	7.80±0.08(bc)	12.65±0.14(f)	1.37±0.01(ab)	1.22±0.04(e)	84.76±0.13(b)
15C (I)	8.59±0.10(a)	13.75±0.33(de)	1.43±0.03(ab)	1.33±0.03(cde)	83.49±0.13(c)
(6)	6.94±0.08(e)	13.60±0.35(e)	1.42±0.01(ab)	1.33±0.06(cde)	83.65±0.13(c)
(12)	7.67±0.06(cd)	13.58±0.17(e)	1.40±0.03(ab)	1.35±0.07(cde)	83.67±0.18(c)
20C (I)	8.87±0.01(a)	14.38±0.28(c)	1.46±0.03(ab)	1.38±0.04(cd)	82.78±0.08(d)
(6)	6.62±0.04(fg)	14.66±0.27(bc)	1.45±0.02(ab)	1.42±0.06(bc)	82.47±0.11(e)
(12)	7.78±0.10(bc)	14.20±0.14(cd)	1.41±0.03(ab)	1.40±0.07(bc)	82.99±0.16(d)
25C (I)	7.69±0.30(bcd)	15.12±0.33(ab)	1.50±0.13(a)	1.60±0.03(a)	81.78±0.11(fg)
(6)	6.68±0.17(efg)	15.35±0.21(a)	1.50±0.01(a)	1.53±0.10(ab)	81.62±0.10(g)
(12)	7.48±0.07(d)	15.02±0.25(ab)	1.48±0.01(a)	1.53±0.03(ab)	81.97±0.21(f)

^a Figures in the same column sharing a common letter in the paranthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

The spaghetti's moisture contents decreased to some extent significantly ($p < 0.05$) during the storage period so all the evaluations were given in dry weight basis. Protein contents of spaghetti's increased significantly by the addition of cowpea flour. The mean values of protein contents of spaghetti's containing 25, 20, 15, 10 and 0% cowpea flour were 14.87; 13.81; 13.30; 12.95 and 11.65% respectively after production.

The addition of the cowpea flour increased the ash and crude oil contents of spaghetti samples, whereas a significant decrease was observed in the carbohydrate content ($p < 0.05$).

Table 3.6. Composition of 10% cowpea flour added spaghetti samples^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
10U (I)	8.86 \pm 0.08(a)	12.99 \pm 0.58(a)	1.41 \pm 0.04(a)	1.27 \pm 0.01(bc)	84.33 \pm 0.17(c)
(6)	7.13 \pm 0.10(f)	13.05 \pm 0.35(a)	1.40 \pm 0.01(a)	1.28 \pm 0.01(bc)	84.27 \pm 0.11(cd)
(12)	8.15 \pm 0.07(d)	12.91 \pm 0.16(a)	1.37 \pm 0.01(a)	1.27 \pm 0.04(bc)	84.45 \pm 0.14(bc)
10G (I)	8.46 \pm 0.01(bc)	13.05 \pm 0.29(a)	1.42 \pm 0.04(a)	1.33 \pm 0.03(ab)	84.02 \pm 0.08(d)
(6)	7.12 \pm 0.13(f)	12.97 \pm 0.18(a)	1.40 \pm 0.02(a)	1.28 \pm 0.04(b)	84.35 \pm 0.07(c)
(12)	8.08 \pm 0.06(d)	12.93 \pm 0.18(a)	1.37 \pm 0.01(a)	1.25 \pm 0.04(bc)	84.45 \pm 0.14(bc)
10F (I)	8.42 \pm 0.04(c)	12.91 \pm 0.13(a)	1.40 \pm 0.01(a)	1.42 \pm 0.04(a)	84.27 \pm 0.11(cd)
(6)	6.71 \pm 0.13(g)	12.95 \pm 0.23(a)	1.39 \pm 0.02(a)	1.23 \pm 0.08(bc)	84.43 \pm 0.10(bc)
(12)	7.87 \pm 0.04(e)	12.88 \pm 0.17(a)	1.38 \pm 0.01(a)	1.24 \pm 0.06(bc)	84.50 \pm 0.14(abc)
10C (I)	8.63 \pm 0.16(b)	12.86 \pm 0.26(a)	1.40 \pm 0.04(a)	1.25 \pm 0.04(bc)	84.49 \pm 0.10(abc)
(6)	6.76 \pm 0.06(g)	12.68 \pm 0.24(a)	1.39 \pm 0.01(a)	1.21 \pm 0.04(c)	84.72 \pm 0.11(ab)
(12)	7.80 \pm 0.08(e)	12.65 \pm 0.14(a)	1.37 \pm 0.01(a)	1.22 \pm 0.04(bc)	84.76 \pm 0.13(a)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.7. Composition of 15% cowpea flour added spaghetti samples^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
15U (I)	8.24 \pm 0.08(c)	12.93 \pm 0.10(b)	1.43 \pm 0.03(a)	1.44 \pm 0.06(a)	84.20 \pm 0.07(b)
(6)	6.79 \pm 0.16(e)	13.40 \pm 0.21(ab)	1.42 \pm 0.02(a)	1.40 \pm 0.06(a)	83.78 \pm 0.04(a)
(12)	7.71 \pm 0.07(d)	13.09 \pm 0.16(ab)	1.40 \pm 0.03(a)	1.36 \pm 0.06(a)	84.15 \pm 0.21(b)
15G (I)	9.20 \pm 0.14(a)	13.65 \pm 0.27(a)	1.45 \pm 0.02(a)	1.41 \pm 0.03(a)	83.49 \pm 0.11(d)
(6)	7.04 \pm 0.23(e)	13.44 \pm 0.25(ab)	1.44 \pm 0.02(a)	1.40 \pm 0.08(a)	83.72 \pm 0.06(cd)
(12)	7.72 \pm 0.04(d)	13.55 \pm 0.14(ab)	1.41 \pm 0.01(a)	1.37 \pm 0.04(a)	83.67 \pm 0.18(cd)
15F (I)	8.57 \pm 0.04(b)	12.95 \pm 0.07(b)	1.45 \pm 0.07(a)	1.38 \pm 0.03(a)	84.22 \pm 0.04(b)
(6)	7.80 \pm 0.14(d)	13.27 \pm 0.17(ab)	1.45 \pm 0.03(a)	1.38 \pm 0.10(a)	83.90 \pm 0.03(bc)
(12)	7.72 \pm 0.08(d)	13.15 \pm 0.21(ab)	1.42 \pm 0.01(a)	1.34 \pm 0.08(a)	84.09 \pm 0.23(b)
15C (I)	8.59 \pm 0.10(b)	13.75 \pm 0.33(a)	1.43 \pm 0.03(a)	1.33 \pm 0.03(a)	83.49 \pm 0.13(d)
(6)	6.94 \pm 0.08(e)	13.60 \pm 0.35(ab)	1.42 \pm 0.01(a)	1.33 \pm 0.06(a)	83.65 \pm 0.13(cd)
(12)	7.67 \pm 0.06(d)	13.58 \pm 0.17(ab)	1.40 \pm 0.03(a)	1.35 \pm 0.07(a)	83.67 \pm 0.18(cd)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.8. Composition of 20% cowpea flour added spaghetti samples^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
20U (I)	8.32 \pm 0.01(bc)	13.36 \pm 0.71(ab)	1.49 \pm 0.01(a)	1.48 \pm 0.04(abc)	83.67 \pm 0.20(a)
(6)	6.99 \pm 0.16(g)	13.71 \pm 0.24(ab)	1.48 \pm 0.01(a)	1.41 \pm 0.10(bc)	83.40 \pm 0.17(ab)
(12)	8.03 \pm 0.10(d)	13.60 \pm 0.14(ab)	1.48 \pm 0.03(a)	1.42 \pm 0.04(abc)	83.50 \pm 0.14(ab)
20G (I)	8.48 \pm 0.04(b)	13.18 \pm 0.85(b)	1.45 \pm 0.07(ab)	1.58 \pm 0.01(a)	83.79 \pm 0.22(a)
(6)	6.83 \pm 0.17(g)	14.14 \pm 0.23(ab)	1.44 \pm 0.01(ab)	1.55 \pm 0.07(ab)	82.87 \pm 0.06(cde)
(12)	7.91 \pm 0.10(de)	13.78 \pm 0.17(ab)	1.43 \pm 0.03(ab)	1.53 \pm 0.04(abc)	83.26 \pm 0.20(bc)
20F (I)	8.24 \pm 0.06(c)	14.33 \pm 0.28(ab)	1.47 \pm 0.02(ab)	1.52 \pm 0.04(abc)	82.68 \pm 0.08(ef)
(6)	6.47 \pm 0.11(h)	13.92 \pm 0.33(ab)	1.47 \pm 0.03(ab)	1.50 \pm 0.04(abc)	83.11 \pm 0.37(bcd)
(12)	7.56 \pm 0.20(f)	13.90 \pm 0.14(ab)	1.45 \pm 0.01(ab)	1.45 \pm 0.03(abc)	83.20 \pm 0.14(bc)
20C (I)	8.87 \pm 0.01(a)	14.38 \pm 0.28(ab)	1.46 \pm 0.03(ab)	1.38 \pm 0.04(c)	82.78 \pm 0.08(def)
(6)	6.62 \pm 0.04(h)	14.66 \pm 0.27(a)	1.45 \pm 0.02(ab)	1.42 \pm 0.06(abc)	82.47 \pm 0.11(f)
(12)	7.78 \pm 0.10(e)	14.20 \pm 0.14(ab)	1.41 \pm 0.03(b)	1.40 \pm 0.07(bc)	82.99 \pm 0.16(cde)

Table 3.8 continued

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

Table 3.9. Composition of 25% cowpea flour added spaghetti samples with the control sample^a (\pm SD, %, in dry weight basis)

Sample ^b	Moisture	Protein	Crude oil	Ash	CHO ^c
25U (I)	8.48 \pm 0.10(a)	14.69 \pm 0.27(ab)	1.53 \pm 0.04(a)	1.67 \pm 0.04(a)	82.11 \pm 0.08(bc)
(6)	6.14 \pm 0.11(e)	14.55 \pm 0.35(b)	1.50 \pm 0.01(a)	1.62 \pm 0.11(a)	82.33 \pm 0.03(ab)
(12)	7.71 \pm 0.13(c)	14.45 \pm 0.21(b)	1.50 \pm 0.01(a)	1.56 \pm 0.06(a)	82.49 \pm 0.23(a)
25F (I)	8.05 \pm 0.07(b)	14.80 \pm 0.28(ab)	1.47 \pm 0.05(a)	1.67 \pm 0.06(a)	82.06 \pm 0.10(bc)
(6)	6.33 \pm 0.10(e)	15.06 \pm 0.20(ab)	1.49 \pm 0.04(a)	1.65 \pm 0.07(a)	81.80 \pm 0.14(cd)
(12)	7.62 \pm 0.11(c)	14.72 \pm 0.11(ab)	1.46 \pm 0.03(a)	1.57 \pm 0.04(a)	82.25 \pm 0.21(ab)
25C (I)	7.69 \pm 0.30(c)	15.12 \pm 0.33(ab)	1.50 \pm 0.13(a)	1.60 \pm 0.03(a)	81.78 \pm 0.11(cd)
(6)	6.68 \pm 0.17(d)	15.35 \pm 0.21(a)	1.50 \pm 0.01(a)	1.53 \pm 0.10(a)	81.62 \pm 0.10(d)
(12)	7.48 \pm 0.07(c)	15.02 \pm 0.25(ab)	1.48 \pm 0.01(a)	1.53 \pm 0.03(a)	81.97 \pm 0.21(bcd)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, ('I' means initially, '6' means six months later, '12' means twelve months later measured data)

^c Carbohydrate (by difference)

The variance analysis results showed that the difference on the amount of ash contents of the groups of 25, 20, 15, 10, 0% cowpea flour added spaghetties was significantly important ($p < 0.05$) but none of these values exceed the maximum ash content given in spaghetti and the macaroni standard (Anonymous, 1989) which is 1.5% without salt in wet basis and 1.67% for 10% moisture containing spaghetties. Ash content of semolina was 1.08 and it increased up to 1.67% for 25% cowpea flour added samples.

The results of protein, ash, crude oil, carbohydrate contents were not significantly different after six months and one year.

3.4. Color

The colours of the blends of durum semolina and cowpea flour samples and spaghetti samples processed from them were measured in triplicate and the mean values were calculated for each of them (Table 3.10).

Table 3.10. Color measurements of blends of cowpea flour and durum wheat flour before spaghetti production^{a,b,c} (\pm SD)

Sample	<i>L</i>	<i>a</i>	<i>b</i>
10U	85.54 \pm 0.04(bc)	1.63 \pm 0.00(h)	19.68 \pm 0.05(cd)
10G	85.42 \pm 0.16(bcd)	1.72 \pm 0.06(gh)	19.63 \pm 0.23(d)
10F	82.75 \pm 0.62(f)	2.97 \pm 0.42(d)	20.11 \pm 0.23(bc)
10C	85.94 \pm 0.15(ab)	1.28 \pm 0.01(i)	19.39 \pm 0.20(de)
15U	85.13 \pm 0.06(cde)	1.90 \pm 0.05(fgh)	19.50 \pm 0.04(de)
15G	85.03 \pm 0.06(cde)	1.98 \pm 0.07(efg)	19.67 \pm 0.08(cd)
15F	80.48 \pm 0.57(g)	4.06 \pm 0.28(c)	20.29 \pm 0.10(f)
15C	85.96 \pm 0.02(ab)	1.33 \pm 0.01(i)	19.23 \pm 0.21(de)
20U	84.80 \pm 0.28(de)	2.09 \pm 0.11(ef)	19.69 \pm 0.18(cd)
20G	84.56 \pm 1.30(e)	2.25 \pm 0.04(e)	19.62 \pm 0.00(d)
20F	79.70 \pm 0.16(h)	4.44 \pm 0.06(b)	20.39 \pm 0.09(ab)
20C	88.85 \pm 0.08(ab)	1.30 \pm 0.01(i)	19.11 \pm 0.33(e)
25U	84.89 \pm 0.18(de)	2.17 \pm 0.06(ef)	19.15 \pm 0.16(e)
25F	77.86 \pm 0.06(i)	5.13 \pm 0.00(a)	20.65 \pm 0.29(a)
25C	85.93 \pm 0.29(ab)	1.28 \pm 0.02(i)	19.37 \pm 0.45(de)
S	86.24 \pm 0.30(b)	1.13 \pm 0.01(i)	19.67 \pm 0.01(cd)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations

^c *L* (brightness, 100=white; 0=black), *a* (+, red; -, green) and *b* (+, yellow; -, blue)

When the statistical analysis was carried out in blends among groups according to the amount of cowpea flour addition as 25, 20, 15, 10 and 0%; the mean '*L*' values for the groups were found 82.89, 83.73, 84.16, 84.91 and 86.34 respectively. As it can be seen the brightness increased while the amount of cowpea flour added was decreased. But the values was not significantly different ($p>0.05$) because the standard deviation within the groups were high. For '*a*' values the mean values were 1.13, 1.90, 2.32, 2.52, 2.86 and for '*b*' the mean values were 19.67, 19.67, 19.70, 19.70, 19.72 and for the same reason there was no significant difference between groups for them.

When the statistical analysis was carried out among groups according to the process type of cowpea flour production unprocessed, fermented, germinated, cooked cowpea flours added in the different amounts; the mean values of '*L*' become as 85.09, 80.20, 85.01 and 85.92 respectively and 86.34 for spaghetti blend without cowpea. This time, there was a significant difference between fermented cowpea flour added samples and the others ($p<0.05$). The situation was similar for '*a*' and '*b*' values that there was a significant difference between the fermented cowpea flour added group and the others.

The color values of spaghetti samples are given in Table 3.11. For spaghetti samples, different from the blends, germinated cowpea flour added spaghetti samples had '*L*' values nearer to the fermented cowpea flour added samples. The addition of cowpea flour generally resulted in significantly darker and more reddish products ($p<0.05$). The production of dark pigments and breakdown of carbohydrates that facilitate Maillard browning reactions during drying may have occurred during fermentation and germination (Prinyawiwatkul et al., 1993). Organic acids produced as a result of sugar metabolism by some microorganisms may also contribute to the darkening of the color (Beuchat, 1976).

In literature, the color of germinated-seed akara was slightly darker and redder than that of the control (Nnanna et al., 1990).

Table 3.11. Color measurements of spaghetti samples just after production^{a,b,c} (\pm SD)

Sample Type	<i>L</i>	<i>a</i>	<i>b</i>
10U	50.38 \pm 0.75(b)	6.98 \pm 0.07(gh)	22.72 \pm 0.19(cd)
10G	48.48 \pm 1.22(d)	7.29 \pm 0.10(fg)	22.77 \pm 0.27(cd)
10F	46.59 \pm 0.43(e)	9.76 \pm 0.20(c)	21.72 \pm 0.16(e)
10C	53.54 \pm 0.95(a)	6.56 \pm 0.01(i)	24.23 \pm 0.16(a)
15U	45.97 \pm 0.83(e)	8.02 \pm 0.34(e)	22.48 \pm 0.58(cd)
15G	46.60 \pm 0.21(e)	8.05 \pm 0. 14(e)	22.85 \pm 0.13(c)
15F	39.41 \pm 0.18(f)	11.23 \pm 0.13(b)	19.50 \pm 0.10(f)
15C	48.71 \pm 0.37(cd)	7.67 \pm 0.03(ef)	23.56 \pm 0.17(b)
20U	51.17 \pm 0.64(b)	7.70 \pm 0.27(ef)	22.30 \pm 0.19(d)
20G	48.98 \pm 1.32(cd)	8.69 \pm 0.02(d)	22.29 \pm 0.30(d)
20F	40.05 \pm 0.52(f)	11.61 \pm 0.27(ab)	18.39 \pm 0.31(g)
20C	47.06 \pm 0.33(e)	7.54 \pm 0.25(f)	22.45 \pm 0.42(cd)
25U	49.98 \pm 0.18(bc)	8.59 \pm 0.11(d)	22.57 \pm 0.14(b)
25F	40.26 \pm 1.42(f)	11.93 \pm 0.50(a)	18.78 \pm 0.39(g)
25C	51.36 \pm 0.82(b)	7.55 \pm 0.29(f)	24.14 \pm 0.28(a)
S	50.55 \pm 0.65(b)	6.75 \pm 0.01(hi)	22.50 \pm 0.00(cd)

^a Figures in the same column sharing a common letter in the paranthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations

^c *L* (brightness, 100=white; 0=black), *a* (+, red; -, green) and *b* (+, yellow; -, blue)

3.5. Sensory Analysis of Textural Attributes and Total Organic Matter (TOM)

TOM (Total organic matter) value of a spaghetti sample is an important quality criteria. It was determined from the washing water of the cooked spaghetti. Total stickiness, firmness and bulkiness values were evaluated with TOM value of a sample .

The data of textural evaluations and total organic matter (TOM) values are presented in Table 3.12. Generally, cowpea added samples had acceptable textural properties and good TOM values. Interestingly, according to the results in Table 3.12, when the amount of unprocessed and germinated cowpea flours increased, the TOM values

decreased. The reason of this might be explained by the increased amount of proteins promoting the building up of a diffused and coagulated protein network capable of entrapping starch granules and preventing them from leaching from pasta during cooking. However, for the sample with cooked cowpea flour, the increased amount of cowpea addition resulted in increased TOM values. This can be explained by the previous denaturation of proteins during the preparation of cowpea flour prevented the later network formation.

The samples with fermented cowpea flour had gained the most unacceptable scores with respect to TOM values. The breakdown of large molecules to smaller fragments during fermentation may increase the TOM values. The textural properties of the samples with the cooked cowpea flour were relatively poor as compared to other samples. The cooking process during the production of cooked cowpea flour might adversely affect the functional parameters measured during the textural evaluation.

As the amount of cowpea added to a spaghetti was decreasing from 25% to 0% the mean values of TOM data were 3.05, 2.45, 2.00, 2.47 and 1.81 respectively. For unprocessed, fermented, germinated, cooked and no cowpea added samples the mean values were 2.14, 2.84, 1.94, 2.77 and 1.81 respectively. The mean values seem to increase with increasing cowpea flour addition amount and the germinated cowpea flour added samples had the best results after the sample without cowpea flour. But the significance of these were low statistically ($p > 0.05$)

According to the sensory analysis of texture results, there was significant difference ($p > 0.05$) between bulkiness values of the cooked and fermented cowpea added spaghetties and 100% semolina spaghetti. There was no significant difference ($p > 0.05$) in other test values. Sometimes addition of cowpea flour had positive effect on spaghetti, but significance level was low.

TOM and sensory analysis of texture results showed that cowpea flour addition to normal semolina in the spaghetti samples is possible without affecting the acceptability values given above. From those types, the best types of cowpea flour seemed to be the germinated and unprocessed ones.

Table 3.12. Sensory analysis of textural attributes data and total organic matter (TOM) values of spaghetti samples as measured just after production ^{a,b}

Sample	Stickiness	Firmness	Bulkiness	Total score	TOM ^c
10U (initial)	little	acceptable	acceptable	48.3±2.9(abc)	2.44±0.03(bc)
(12 m)	little	good	acceptable	48.0± 3.4(abc)	2.55±0.15(bc)
10G (initial)	medium	acceptable	acceptable	48.3±7.6(abc)	2.18±0.03(c)
(12 m)	medium	acceptable	acceptable	50.3±4.5(ab)	2.25±0.21(c)
10F (initial)	too little	good	acceptable	58.3±2.9(a)	2.74±0.48(b)
(12 m)	too little	good	acceptable	56.3±2.0(a)	2.74±0.48(b)
10C (initial)	too little	acceptable	acceptable	53.3±5.8(ab)	2.44±0.16(bc)
(12 m)	too little	medium	acceptable	52.3±1.9(ab)	2.54±0.48(bc)
15U (initial)	little	acceptable	acceptable	44.0±1.7(cd)	1.81±0.03(de)
(12 m)	little	acceptable	acceptable	43.2±3.9(cd)	1.82±0.08(de)
15G (initial)	little	medium	acceptable	41.7±1.5(cd)	1.94±0.01(d)
(12 m)	little	medium	acceptable	40.3±2.2(cd)	2.01±0.28(d)
15F (initial)	little	acceptable	acceptable	48.3±2.4(abc)	2.05±0.03(d)
(12 m)	too little	acceptable	acceptable	47.9±2.9(abc)	2.80±0.45(b)
15C (initial)	medium	acceptable	medium	38.3±5.8(e)	2.21±0.04(c)
(12 m)	medium	acceptable	medium	33.3±2.6(f)	2.28±0.24(c)
20U (initial)	little	acceptable	acceptable	55.7±4.0(a)	1.59±0.03(e)
(12 m)	little	good	acceptable	55.3±2.1(a)	1.65±0.28(e)
20G (initial)	little	acceptable	too little	55.0±5.0(a)	1.71±0.01(e)
(12 m)	little	acceptable	too little	53.3±2.5(a)	1.74±0.16(e)
20F (initial)	little	medium	too little	50.0±10.0(abc)	3.87±0.08(a)
(12 m)	too little	medium	acceptable	45.3±2.9(c)	3.74±0.32(a)
20C (initial)	too little	medium	acceptable	41.7±2.9(cd)	2.71±0.01(b)
(12 m)	too little	medium	acceptable	40.3±2.9(cd)	2.74±0.23(b)
25U (initial)	little	acceptable	too little	55.0±5.0 (a)	2.74±0.01(b)
(12 m)	little	acceptable	too little	54.4±1.9(a)	2.75±0.12(b)
25F (initial)	little	acceptable	acceptable	53.3±2.9 (ab)	2.71±0.06(b)
(12 m)	too little	acceptable	acceptable	52.0±2.9(ab)	2.74±0.16(b)
25C (initial)	medium	acceptable	medium	43.3±5.8(cd)	3.70±0.04(a)
(12 m)	medium	acceptable	medium	41.1±3.9(cd)	3.54±0.08(a)
S (initial)	little	acceptable	too little	56.7±7.6(a)	1.81±0.13(de)
(12 m)	little	acceptable	too little	56.3±5.9(a)	1.83±0.28(de)

^a Figures in the same column sharing a common letter in the paranthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations, “12 m” means after 12 months

^c Grams per 100 g of dry pasta.(>2.1=low quality; 1.4-2.1=good quality;<1.4=very good quality)

3.6. Phytic Acid Determination

Cooking, fermentation and germination decreased the phytic acid amounts of the samples. The amount of phytic acid originally present in the control unprocessed

cowpea flour was found to be 677 mg/100 g. The phytic acid content of cowpea flour obtained in the previous studies ranged from 290-992 mg/100 g (Lasztity, and Lasztity, 1990; Kumar and Venkatasgmun, 1978; Ene-Obong, 1995; Giami et al., 2001). The amount of phytic acid shows significant variations ($p>0.05$) among the varieties (Lasztity, and Lasztity, 1990). Research has shown that processes like germination (Nnanna et al., 1990), fermentation (Zamora and Fields, 1979) and cooking (Prinyawiwatkul et al., 1996a) of cowpeas do not only improve nutritional quality, flavor and functional properties but also reduce undesirable factors including phytic acid. Processes applied in this research reduced the phytic acid content of the cowpea flour (Table 3.13) by approximately 13.29-19.05%.

Table 3.13. Phytic acid content of cowpea flours ^a(\pm SD, in dry weight basis)

Cowpea Flour Type	PA ^b	% loss during the process
Unprocessed	677 \pm 8(a)	-
Germinated(1) ^c	583 \pm 7(b)	13.88 \pm 0.41(e)
Germinated(2) ^c	557 \pm 4(cd)	17.73 \pm 0.10(b)
Germinated(3) ^c	553 \pm 7(d)	18.32 \pm 0.10(ab)
Fermented(1) ^c	587 \pm 9(b)	13.29 \pm 0.30(e)
Fermented(2) ^c	564 \pm 8(cd)	16.69 \pm 0.16(c)
Fermented(3) ^c	548 \pm 11(d)	19.05 \pm 0.35(a)
Cooked	574 \pm 4(b)	15.21 \pm 0.51(d)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

^b PA: Phytic acid (mg/100g)

^c Numbers in the parenthesis shows the number of the days of the process

The effect of storage on the phytic acid amount of spaghetti samples are given in Table 3.14. While the initial amount of phytic acid was 220.4 mg/100 g in S, it changed between 242.2-300.0 mg/100 g in other samples. It is seen from Table 3.14 that twelve months of storage period cause a small decrease on the phytic acid content of samples about 1%. For this storage period, the loss of phytic acid in some of the samples were significantly important ($p>0.05$).

Table 3.14. The effect of storage on the phytic acid amount of spaghetti samples^{a,c} (\pm SD, in dry weight basis)

Sample	PA ^b	PA ^b	%loss ^d	PA ^b	%loss ^d
	(initial)	(six months later)		(twelve months later)	
10U	254.6 \pm 0.6(f,A)	253.9 \pm 0.4(f,A)	0.29 \pm 0.10(a)	252.1 \pm 1.8(g,A)	0.98 \pm 0.15(b)
10G	246.4 \pm 0.8(g,A)	245.8 \pm 0.3(g,A)	0.28 \pm 0.13(a)	242.3 \pm 0.1(h,B)	1.66 \pm 0.25 (a)
10F	242.9 \pm 0.1(h,A)	241.9 \pm 1.3(h,A)	0.40 \pm 0.15(a)	240.6 \pm 0.7(i,A)	0.95 \pm 0.17(b)
10C	242.2 \pm 0.1(h,A)	241.4 \pm 1.0(h,A)	0.32 \pm 0.08(a)	239.3 \pm 0.1(i,B)	1.2 \pm 0.13(b)
15U	264.5 \pm 0.3(e,A)	263.7 \pm 0.9(e,A)	0.31 \pm 0.05(a)	261.0 \pm 0.9(f,B)	1.32 \pm 0.10(ab)
15G	255.0 \pm 0.7(f,A)	253.8 \pm 1.0(f,A)	0.45 \pm 0.21(a)	251.4 \pm 1.1(g,B)	1.41 \pm 0.20(a)
15F	253.5 \pm 0.3(f,A)	252.4 \pm 0.3(f,A)	0.46 \pm 0.11(a)	251.7 \pm 0.6(g,A)	0.71 \pm 0.10(c)
15C	253.2 \pm 0.1(f,A)	252.5 \pm 0.8(f,A)	0.29 \pm 0.09(a)	251.6 \pm 1.3(g,A)	0.63 \pm 0.32(c)
20U	281.6 \pm 0.3(b,A)	280.7 \pm 1.0(b,A)	0.30 \pm 0.09(a)	278.3 \pm 0.8(b,B)	1.17 \pm 0.26(b)
20G	264.2 \pm 1.1(e,A)	263.3 \pm 0.1(e,A)	0.33 \pm 0.10(a)	261.3 \pm 0.1(f,B)	1.1 \pm 0.18(b)
20F	268.4 \pm 0.8(d,A)	267.6 \pm 0.3(d,A)	0.30 \pm 0.12(a)	265.4 \pm 0.9(e,B)	1.12 \pm 0.21(b)
20C	263.1 \pm 0.6(e,A)	261.9 \pm 1.1(e,A)	0.45 \pm 0.09(a)	260.4 \pm 0.3(f,B)	1.03 \pm 0.20(b)
25U	300.0 \pm 1.4(a,A)	298.9 \pm 0.1(a,A)	0.36 \pm 0.08(a)	297.6 \pm 0.1(a,B)	0.8 \pm 0.15(c)
25F	280.5 \pm 2.8(b,A)	279.5 \pm 1.4(b,A)	0.35 \pm 0.09(a)	276.5 \pm 0.4(c,B)	1.43 \pm 0.20(a)
25C	275.9 \pm 1.4(c,A)	275.1 \pm 0.3(c,A)	0.30 \pm 0.08(a)	273.9 \pm 0.4(d,A)	0.72 \pm 0.40(c)
S	220.4 \pm 0.1(i,A)	219.8 \pm 0.7(i,A)	0.28 \pm 0.10(a)	217.8 \pm 0.1(j,B)	1.18 \pm 0.30(b)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level. Figures in the same raw sharing a common capital letter in the parenthesis are not significantly different at 0.05 level.

^b PA: Phytic acid(mg/100g)

^c See Table 2.1, for abbreviations

^d % loss was calculated based on initial PA

The percentage of addition of cowpea flour was found to be significantly important, and so when the amount of supplement was increased the amount of phytic acid also increased. The initial amount of phytic acid which was 220.0 mg/100g sample of the control spaghetti increased to 300.0, 280.5 and 275.9 mg/100g in the 25U, 25F and 25C samples respectively.

The effects of a chosen process on the amount of phytic acid are different from each other. Ologhobo and Fetuga (1984) found germination caused the most

pronounced effect on the loss of phytic acid with respect to the processes of cooking, autoclaving and soaking. According to a study of Ibrahim et al. (2002) among the processes of fermentation, germination, soaking and cooking of cowpea fermentation by fungi or bacteria caused the highest reduction (47.36%) in phytic acid but it was reported that more researches were needed to optimize fermentation conditions to modify cowpea composition without increasing tannins. In the same study the percent loss of phytic acid was 18.06% for 24 h mold fermented cowpea samples, 17.4% for 24 h germinated cowpeas, 6.8% for cooked cowpeas, 11.9% for pressure cooked cowpeas, 20.7% for soaked and cooked cowpeas and 32.8% for soaked and pressure cooked cowpeas. In a study (Yılmaz and Ünal, 1993) the phytic acid amount of semolina were observed to decrease about 23.44% during spaghetti production probably because of phytase enzyme activity.

In this study, there was no significant difference ($p>0.05$) between these processes of germination, fermentation, cooking in the extend of loss of the amount of phytic acid in cowpea flours, on the other hand, in spaghetti samples the amount of phytic acid showed little variations according to the type of cowpea flour added.

3.7. Total Antioxidant Capacity (TAC) Determination

Total antioxidant capacity (TAC, $\mu\text{mol trolox equivalent/g}$) of cowpea flours and samples with added cowpea flour are given in Table 3.15 and 3.16. The samples with 20% cowpea flour added spaghetti samples were analysed for their TAC values to investigate the effect of cowpea flour addition on total antioxidant capacity. Cooking the cowpea flour resulted in a decrease in the TAC values, whereas germination resulted in increased TAC values as compared to unprocessed control flour. The addition of cowpea flour increased the TAC values of spaghetti samples as compared to control sample (i.e. 100 % semolina). Some compounds, which might be bound to the cell walls, were released during cooking or they might be bound to insoluble fraction of other compounds formed during cooking of seeds. In germinated seeds, data was in agreement with a well-known fact that the biosynthesis of some compounds including the ones that have antioxidant effect like vitamin C take place during germination (Fernandez-Orozco, and Piskula, 2003). In a study examining four cultivars of lentil showed that antioxidant capacity of these lentil seeds formed by low molecular weight antioxidants, mainly by phenolic compounds as well as by

tocopherols, reduced glutathione and ascorbate (Fernandez-Orozco, and Piskula, 2003).

Table 3.15. Total antioxidant capacity (TAC, $\mu\text{mol trolox equivalent/g}$) of cowpea flours (\pm SD, in dry weight basis)^a

Cowpea flour type	TAC
Unprocessed	26.89 \pm 0.04(b)
Germinated	28.63 \pm 0.03(a)
Fermented	26.78 \pm 0.01(c)
Cooked	19.40 \pm 0.03(d)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level.

Total antioxidant capacities (TAC) of the cowpea flour samples showed that while germination was having a positive effect on the TAC, fermentation was not affective and cooking process decreased it. After spaghetti production, TAC values obtained for 20% cowpea added spaghetti samples and spaghetti without cowpea. The effect of storage on the TAC of spaghetti samples were also determined (Table 3.16).

Table 3.16. Total antioxidant capacity (TAC, $\mu\text{mol trolox equivalent/g}$) of spaghetti samples (\pm SD, in dry weight basis)^{a,b}

Spaghetti type	TAC	TAC	TAC
	(initial)	(after 6 months)	(after 12 months)
20U	31.88 \pm 0.01(b,A)	28.36 \pm 0.01(b,B)	26.55 \pm 0.01(b,C)
20G	34.51 \pm 0.01(a,A)	28.48 \pm 0.01(a,B)	26.73 \pm 0.03(a,C)
20F	31.16 \pm 0.01(c,A)	23.06 \pm 0.03(d,B)	20.01 \pm 0.04(d,C)
20C	27.53 \pm 0.03(d,A)	25.70 \pm 0.07(c,B)	22.16 \pm 0.09(c,C)
S	24.49 \pm 0.03(e,A)	20.98 \pm 0.01(e,B)	17.93 \pm 0.02(e,C)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level. Figures in the same raw sharing a common capital letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations

It was observed that storage caused a decrease in TAC values of all samples studied (Table 3.16).

The TAC values of samples decreased significantly in the period of one year (mean values were initially: 29.91, after six months: 25.32, after a year: 22.41 $\mu\text{mol trolox equivalent/g}$)

3.8. Microbial Analysis of Spaghetti Samples

According to spaghetti and macaroni standard (Anonymous, 1989) APC of samples should not be greater than 100 000 cfu/g (5 log cfu/g), and mold and yeast number should be less than 500 cfu/g (2.699 log cfu/g).

Spaghetti production involves a controlled drying step at warm temperatures which, if not properly controlled, enables the extensive growth of microorganisms (Swartzentruber et al., 1982). Most of the microbiological studies of spaghetti products have focused on pathogen growth during production rather than on the general microbiological quality. Since spaghetti is generally regarded as a microbiologically safe product, as it has relatively low water activity (0,50) (<http://www.lsbu.ac.uk/water/activity.html>). Swartzentruber et. al. (1982) found that the geometric means of aerobic plate counts for spaghetti and noodle products were 520 and 1400 per g, respectively; means of yeast and mold counts were 72 per g for spaghetti and 100 per g for noodles. These values were within the safe limits (Rayman et al., 1981). During cowpea flour production, some processes especially fermentation may lead to contamination by pathogens which can then survive for some period.

In this study, aerobic plate counts (APC) (Table 3.17) and mold and yeast counts (Table 3.18) were conducted for the control and enriched spaghetti samples containing 20% differently processed cowpea flours over one year of storage period and compared with the standarts.

The results showed that aerobic plate count (APC) of the samples were not significantly different from each other ($p < 0.05$) (Table 3.17) and there was no significant increase during the storage period for the first six months and some

decrease in APC values was observed for the last six months. APC results show that addition of cowpea flour did not affect microbiological safety of samples. For cereals and legumes soaking and cooking processes were both found to leach out or decrease some undesirable factors and reduce the in-situ microbial contaminants especially nonsporulating bacteria and molds. In general, the fermentation increased the shelf life of cereals and legumes (Wang and Hesseltine, 1981).

Table 3.17. Aerobic plate counts of the spaghetti samples enriched with 20 % cowpea flour (log CFU/g)^{a, b}

Spaghetti	Initial	after 6 months	after 12 months
20U	2.41±0.014(a,A)	2.42±0.071(a,A)	2.20±0.010(a,B)
20G	2.41±0.007(a,A)	2.42±0.057(a,A)	2.20±0.050(a,B)
20F	2.41±0.028(a,A)	2.42±0.007(a,A)	2.19±0.005(a,B)
20C	2.41±0.099(a,A)	2.42±0.028(a,A)	2.20±0.015(a,B)
S	2.41±0.028(a,A)	2.41±0.127(a,A)	2.15±0.026(b,B)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level. Figures in the same raw sharing a common capital letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations

Table 3.18. Mold and yeast count of the spaghetti samples enriched with 20 % cowpea flour (log of CFU/g)^{a, b}

Spaghetti	Initial	after 6 months	after 12 months
20U	1.84±0.014(a,A)	1.86±0.002(b,A)	1.75±0.022(a,B)
20G	1.85±0.007(a,B)	1.88±0.003(a,A)	1.70±0.030(b,C)
20F	1.85±0.007(a,B)	1.89±0.014(a,A)	1.75±0.035(a,C)
20C	1.84±0.004(a,B)	1.85±0.008(b,A)	1.73±0.011(ab,C)
S	1.79±0.016(b,B)	1.82±0.014(c,A)	1.70±0.014(b,C)

^a Figures in the same column sharing a common letter in the parenthesis are not significantly different at 0.05 level. Figures in the same raw sharing a common capital letter in the parenthesis are not significantly different at 0.05 level.

^b See Table 2.1, for abbreviations

The mold and yeast count of the control and cowpea flour supplemented spaghetti samples increased during storage for the first six months and decreased for the last six months significantly ($p < 0.05$) (Table 3.18). The APC and mold and yeast counts of the spaghetti samples were both below the safety limit of the spaghetti standards, which are 5 and 2.699 log CFU/g, respectively (Anonymous, 1989).

3.9. *In Vitro* Protein Digestibility

The *in vitro* protein digestibility (IVPD) values of spaghetti samples studied are given in Table 3.19. Results show that adding cowpea flour to spaghetti at 20 % did not change the IVPD values of the sample ($p < 0.05$). It is also seen from Table 3.19 that storage of one year at room temperature ($\sim 25^{\circ}\text{C}$) did not affect the IVPD of the samples ($p < 0.05$). It was observed that, during IVPD analysis, the pH dropped suddenly for the first two minutes and then decreased more slowly after 4 minutes incubation as possibly the substrate to enzyme ratio declines. During cereal-enzyme incubation, hydrolysis occurs and amino acids are released from the peptide chain, resulting in decrease on pH, a marker for increased protein digestibility (Dahlin and Lorenz, 1993). The IVPD results were between 78.334 and 79.420 %, which were not significantly different from each other ($p > 0.05$).

The results of studies on the effect of germination on *in vitro* protein digestibility were different from each other in the literature. Nnanna and Phillips (1989) examined the effect of germination, decortication and cooking (in water at 100°C , 1 h) on amino acid composition, protein nutritional quality, and vitamin content of cowpeas. The treatments had no effect on essential amino acids other than tryptophan which increased slightly in some treatments. Germination did not improve *in vitro* digestibility, and other measures of protein quality were little affected by the treatment. It was stated that IVPD of cowpea was not improved significantly neither by germination nor by decortication but was improved by cooking (Nnanna and Phillips, 1989).

Njintang et al. (2001) revealed that protein digestibility of bean flours increased with germination and drying temperature. Fermentation and germination significantly ($p > 0.05$) increased protein digestibility of fluted pumpkin seed flours (Giarni, 1999). Casagrandi et al., (1999) found that the addition of pigeon pea flour to spaghetti

decreased protein digestibility by approximately 4 %. However, Rayas-Duarte et al., (1996) found that spaghetti made from lupin flours retain their overall digestibility compared to the control. Giami et al. (2001) found that boiling the African breadfruit seeds for 5-20 minutes improved protein digestibility. Giami (1999) also observed that fermentation and germination significantly ($p>0.05$) lowered polyphenol and phytic acid contents but increased protein digestibility of fluted pumpkin seed flours.

Table 3.19. *In vitro* protein digestibility of spaghetti samples enriched with 20 % cowpea flour (\pm SD, %, in dry weight basis)^{a,b}

Spaghetti	Initial	after 6 months	after 12 months
20U	78.51 \pm 0.57	79.33 \pm 0.88	78.35 \pm 0.56
20G	79.09 \pm 0.50	80.01 \pm 0.74	78.50 \pm 0.80
20F	78.33 \pm 0.94	78.88 \pm 0.88	78.28 \pm 1.05
20C	79.42 \pm 0.82	80.23 \pm 0.97	79.13 \pm 0.75
S	78.93 \pm 0.99	79.44 \pm 0.44	79.40 \pm 0.60

^a *In vitro* protein digestibility values of spaghetti samples were not statistically different at 0.05 level.

^b See Table 2.1, for abbreviations

As the major purpose of germination was to reduce the flatulence potential of cowpea, the effect of germination and decortication on starch and protein digestibility of cooked cowpea and on flatulence production in rats were examined (Nnanna and Phillips, 1989). While germination at 30°C for 24 h was reducing flatus production from 2.8 to 0.64 ml/g cowpea consumed compared to 0.15 ml/g for cowpea-free maintenance diet it also increased *in vivo* protein and starch digestibility significantly from 82 to 86% and 96.6 to 98%, respectively.

The IVPD results of enriched spaghetti were comparable to the previous studies, where IVPD values were found as 82.41% for the normal macaroni samples (Pınarlı, Öner and İbanoğlu, 2004) and apparent protein digestibility of cowpeas was said to be between 65.0-80.2% (Bressani, 1993).

3.10. Differential Scanning Calorimetry

The objective of the DSC measurements was to determine changes in starch gelatinization after the addition of different types of cowpea flour to spaghetti samples.

Gelatinized starch often plays an important role in determining the structural, textural and physical properties of many foods, including bakery products. Differential scanning calorimetry (DSC), initially developed as a tool to characterize thermal transitions in polymers, has now become a standard method for measuring the thermal properties of food and biopolymers. Starch gelatinization involves disaggregation of starch granules within an aqueous medium at appropriate temperature. The temperature for maximum gelatinization ranges between 50 and 70°C, depending upon the type of starch and its origin. The gelatinization process goes through some non-equilibrium and complex transitions, such as glass transition, hydration and swelling of the amorphous regions, the melting of crystallites within the granule, simultaneous crystallization on heating and the formation of starch-lipid complexes (Marcotte et al., 2004).

Results revealed that there were two endothermic transitions during DSC analysis of the samples (Table 3.20 and 3.21). It has been stated that the first endothermic transition relates to the gelatinization of starch at excess water and the second one to the possible denaturation of proteins and reversible dissociation of amylose-lipid complexes (Lupano and Gonzales, 1999; Zweifel et al., 2000).

Data for the first melting transition of the samples is in Table 3.20. The temperatures of gelatinization onset (T_0) were changing from 59.9°C to 61.7°C and the melting temperatures were between 67.5-69.9°C. The T_{01} (the onset temperature of the first peak) values obtained in our study were lower than the values of T_{01} reported (~76°C) for cowpea flour (Henshaw et al., 2003) and higher than that (62.5°C) of the wheat starches (Choi and Kerr, 2004). The difference may be due to variations on the amount and type of starches in samples. Also, the starch transformations are said to be primarily governed by temperature, moisture and time conditions (Zweifel et al.,

2000). The results of T_{o1} values in this study are comparable to those of a previous study where the starch gelatinization onset temperature shifted from 67°C for starch to 72°C for 25% cowpea flour that contained 12-15% starch (Okechukwu et al., 1991). When wheat starch was isolated, there observed one definite endothermic transition in DSC analysis (Choi and Kerr, 2004).

Table 3.20. Thermal properties of spaghetti samples just after production enriched with 20 % cowpea flour obtained from the first endothermic peak of DSC (\pm SD, in dry weight basis)^{a,b}

Sample	T_{o1} (°C)	T_{p1} (°C)	PHI_1 (J/g°C)	ΔH_1 (J/g)
20U	61.7 \pm 0.28(a)	66.9 \pm 0.14(b)	0.48 \pm 0.02(c)	2.48 \pm 0.01(d)
20G	59.9 \pm 0.14(c)	67.7 \pm 0.13(a)	0.43 \pm 0.01(d)	3.37 \pm 0.02(c)
20F	60.9 \pm 0.14(b)	67.9 \pm 0.09(a)	0.35 \pm 0.04(e)	2.41 \pm 0.02(e)
20C	60.5 \pm 0.21(b)	67.6 \pm 0.14(a)	0.54 \pm 0.01(b)	3.90 \pm 0.03(b)
S	60.6 \pm 0.14(b)	67.5 \pm 0.21(a)	0.61 \pm 0.03(a)	4.21 \pm 0.03(a)

^a Figures in the same raw sharing a common letter in the paranthesis are not significiantly different at 0.05 level.

T_o :onset temperature, T_p :peak temperature, PHI:peak height index ($\Delta H/(T_p-T_o)$), ΔH :enthalpy.

^b See Table 2.1, for abbreviations

The melting entalpy values (ΔH) of the samples were between 2.41-4.21 mW. The entalpy value of spaghetti produced from 100% semolina was the highest and addition of cowpea flour decreased the ΔH values. This may be explained by the increased protein content of samples with cowpea addition, leading to decreased enthalpy values for starch gelatinization (Lundqvist and Eliasson, 2005). A decrease in ΔH_1 values could also mean that the starch in the blend has less available water due to protein competition (Mohamed and Rayas-Duarte, 2003). Studies showed that the gelatinization peak temperature of the starch increased and ΔH decreased in the presence of proteins (Eliasson, 1983). The PHI values given in the Table 3.20 and 3.21 for the first and second peaks indicate the shape and degree of symmetry of the endotherm. A high PHI indicates a narrow transition range (Henshaw et al., 2003). For the first peak, peak hight index (PHI) of the enriched samples were significiantly

lower than that of the control one. PHI was calculated by the formula of $\Delta H / \Delta T$ where ΔT is melting range of samples.

The data for the second peak during DSC analysis are given in Table 3.21. The protein denaturation and dissociation of amylose-lipid complex in the samples started at 78.9-88.9°C and peaked at 87.6-100.4°C. Peak height index (PHI) of enriched samples in the second peak, were significantly higher than that of the control sample. The spaghetti sample enriched with unprocessed cowpea flour had a peak with the lowest T_{o2} (78.9°C) and T_{p2} (90.0°C) with the highest ΔH_2 (enthalpy) (3.86 J/g) among the other samples studied. These values were lower than the values reported for denaturation of cowpea globulins (Henshaw et al., 2003).

Table 3.21. Thermal properties of spaghetti samples just after production enriched with 20 % cowpea flour obtained from the second endothermic peak of DSC(\pm SD, in dry weight basis)^{a,b}

Sample	T_{o2} (°C)	T_{p2} (°C)	PHI ₂ (J/g°C)	ΔH_2 (J/g)
20U	78.9 \pm 0.14(e)	90.0 \pm 0.12(c)	0.35 \pm 0.007(b)	3.86 \pm 0.08(a)
20G	81.2 \pm 0.07(d)	86.9 \pm 0.15(d)	0.31 \pm 0.003(c)	1.80 \pm 0.03(d)
20F	82.2 \pm 0.01(c)	87.6 \pm 0.26(d)	0.52 \pm 0.004(a)	2.79 \pm 0.01(b)
20C	85.9 \pm 0.14(b)	93.8 \pm 0.21(b)	0.26 \pm 0.003(d)	2.02 \pm 0.01(c)
S	88.9 \pm 0.06(a)	100.4 \pm 1.55(a)	0.15 \pm 0.008(e)	1.71 \pm 0.04(e)

^a Figures in the same row sharing a common letter in the parenthesis are not significantly different at 0.05 level.

To:onset temperature, Tp:peak temperature, PHI:peak height index ($\Delta H / (T_p - T_o)$), ΔH :enthalpy.

^b See Table 2.1, for abbreviations

The differences between the results of different studies may result from different compositions as well as different sources. The enthalpies obtained in DSC analysis represent a composite, comprising the balance of heat changes involved with gelatinization of starch, denaturation of proteins and the changes associated with protein-starch interactions (Henshaw et al., 2003) which probably affected the cooking properties of spaghetti.

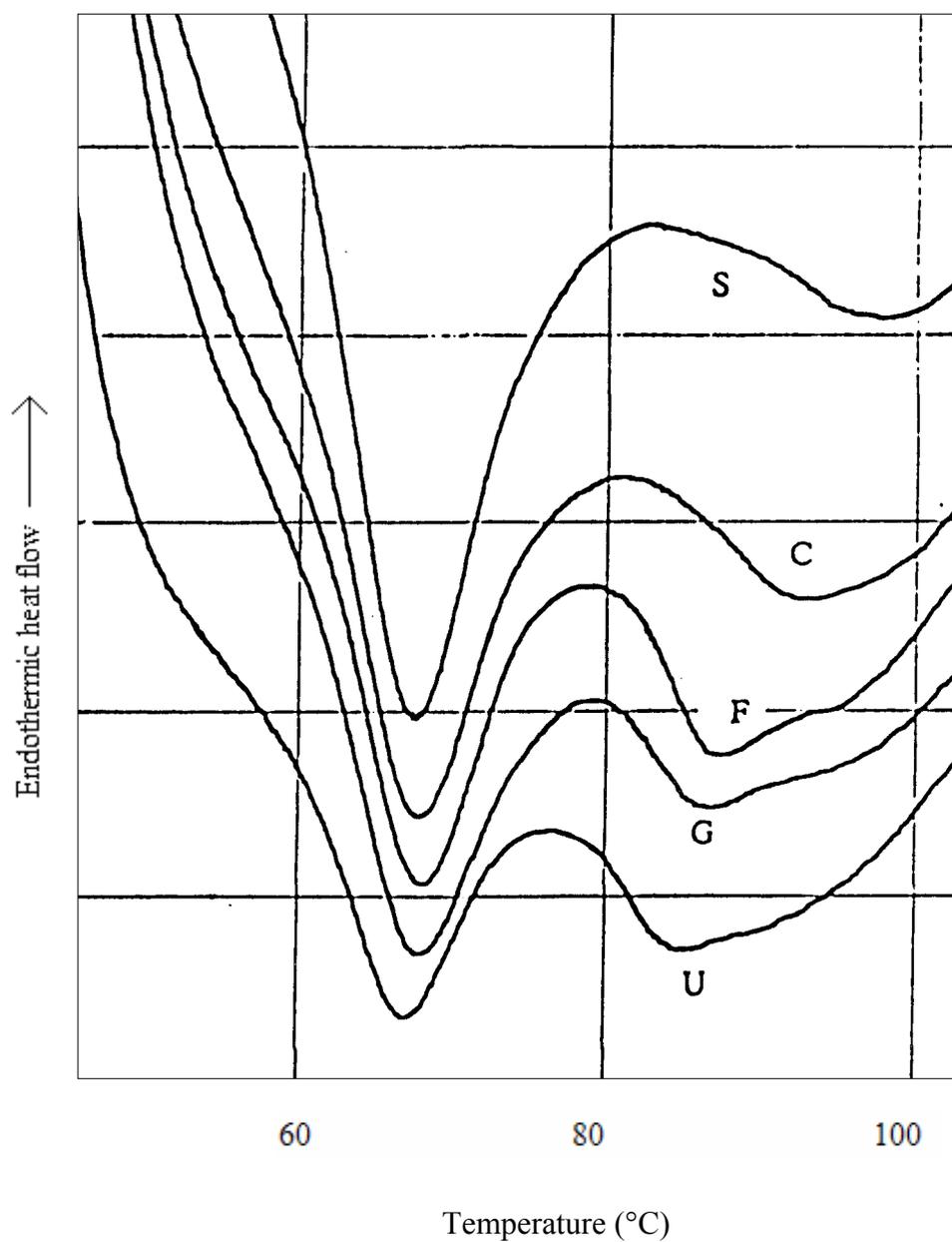


Figure 3.3. DSC thermograms of spaghetti samples enriched with 20% cowpea flour^a
 See Table 2.1, for abbreviations, ΔH :enthalpy (J/g)

The processing applied in this study (i.e. germination, fermentation, cooking) may cause the differences shown. The mechanisms causing these differences for the onset of gelatinization in the samples during thermal analysis require further research.

4. CONCLUSION

1. Cowpea flour was found to have more than two times protein, more than three times ash, one and a half times much crude oil content than semolina. Cooking decreased the protein and ash contents slightly while germination and fermentation caused less variation in protein content comparatively.
2. Addition of 10, 15, 20 and 25% cowpea flour increased the protein content of control spaghetti from 11.65 to 12.95, 13.30, 13.81 and 14.87%, respectively.
3. The colour of spaghetti samples with added cowpea flour especially with fermented cowpea flour were darker and redder than control spaghetti sample with no added cowpea flour.
4. According to sensory analysis of textural attributes of spaghetti samples, most of the total scores of cowpea flour enriched spaghetti samples were not significantly different from the control (produced from 100% wheat semolina) ($p < 0.05$). It can be concluded from the results of this study that processed cowpea flour can be successfully used in spaghetti production up to 25 % cowpea addition. Enriched spaghetti samples had good cooking quality with regard to stickiness, bulkiness, firmness and total organic matter. Compared to other spaghetti samples studied, the sample with cooked cowpea flour had relatively lower textural values and the samples with fermented cowpea flour had higher TOM values.
5. Phytic acid amounts of the cowpea flours decreased by cooking, fermentation and germination processes, however, because cowpea flours have higher amount of phytic acid than semolina, addition of cowpea flour to the spaghetti samples increased the phytic acid content of spaghetti compared to the control spaghetti. Percent loss of phytic acid amount of the samples were determined as 0.28-0.46% for the six months of storage and 0.63-1.43% for the twelve months of storage period.

6. Cooking had a negative effect on the total antioxidant capacity (TAC) of cowpea flours while germination affected the TAC of the samples positively. During storage TAC of spaghetti samples decreased. The mean TAC values of all samples studied were determined as 29.91 initially, 25.32 after six months and 22.41 $\mu\text{mol trolox equivalent/g}$ after one year.
7. It can be concluded from the results of this study that addition of cowpea flours at 20 % level and storage of them for one year did not affect the microbiological safety of samples with respect to food codex (Anonymous, 1989) though mold and yeast counts of samples were significantly higher than that of the control sample.
8. Addition of cowpea flour at 20 % level did not affect *in vitro* protein digestibility of spaghetti samples adversely during the storage period.
9. Spaghetti samples containing cowpea flour had lower starch gelatinization and higher protein denaturation and dissociation of amylose-lipid complex enthalpies than the control spaghetti without addition of cowpea flour. Due to the total enthalpy values, cowpea flour addition increased the need of heat required for cooking of spaghetties except for the spaghetti including cooked cowpea flour which had the same amount of total enthalpy as control spaghetti.
10. Cowpea flour enrichment increased the protein amount and quality of spaghetti without affecting microbial quality and protein digestibility. Total antioxidant capacity (TAC) and concentration of color of spaghetties increased by cowpea flour addition. The spaghetties including processed cowpea flour had less phytic acid amount than the spaghetties with unprocessed cowpea flour. It can be concluded from the results of this study that processed cowpea flour can be successfully used in the spaghetti production. The best types of cowpea flour to be incorporated into spaghetti were proposed to be the unprocessed and than germinated types up to 20% based on the sensory analysis of textural attributes and TOM values.

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PUBLICATIONS

1. Herken, E.N., İbanoğlu, Ş., Öner, M.D. and İbanoğlu, E. The *in vitro* protein digestibility, microbiological quality and gelatinization behaviour of macaroni as affected by cowpea flour addition, *Food Chemistry* (In Press).
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HOBBIES

Reading
Swimming

