

**PHYSICO-CHEMICAL PROPERTIES AND  
SPAGHETTI MAKING QUALITY OF INDIAN  
DURUM WHEAT**

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## SYNOPSIS

Durum wheat (*Triticum durum*) is a tetraploid wheat and a separate species from other commercially grown wheat classes, which is mainly *T. aestivum*, a hexaploid wheat. Durum wheat has unique characteristics, which have made it the most suitable raw material for the production of pasta products such as spaghetti, macaroni, vermicelli etc. Durum wheat represents around 6-8% of the total wheat production in the world. India produces a considerable amount of durum wheat (~2.5 Mt), which is mostly consumed in the domestic market and for the preparation of several Indian traditional dishes. On the other hand, pasta products are becoming increasingly popular not only world wide but also in the Indian subcontinent because of their ready-to-eat convenient form, availability in various shapes and designs, nutritional quality, palatability, and long shelf life. Spaghetti that is the solid cylindrical form of pasta, is the most popular and common variety among the hundreds of shapes of pasta products and is the preferred pasta shape for laboratory evaluation of pasta, because its geometrical form gives the best indication of the intrinsic quality of durum wheat semolina. Although India has the potential to export durum wheat to the world market, demand for Indian durum wheat is also expanding in the domestic market. However, there is little information available regarding various characteristics of Indian durum wheat and their relation to semolina milling and pasta, especially spaghetti-making quality.

In view of the above, the present research work was undertaken to study the following three main objectives:

1. Physico-chemical characteristics,
2. Semolina milling properties, and

### 3. Spaghetti-making quality

of Indian durum wheat varieties in the thesis entitled “Physico-chemical properties and spaghetti making quality of Indian durum wheat”.

The whole write up of the thesis is divided into six sections:

**Section I** includes “**Introduction**”, which provides general information about classification of wheat and position of durum wheat in this classification, statistics of world and Indian wheat production, consumption, and trade, and also durum wheat situation in the world and India, its production, uses, exports, and imports. This section ends with scope and objectives of the work, which covers the need for the study and major objectives of this investigation.

**Section II** covers the “**Review of Literature**” regarding durum wheat, its definition and importance for pasta making, quality aspects of durum wheat that are important in milling properties and semolina production such as test weight, 1000-kernel weight, vitreousness, protein, ash, and moisture contents. The process of semolina milling and quality aspects of semolina such as particle size, moisture content, ash content, color, and protein content are also reviewed. Classification of durum wheat products with focus on pasta products and their nutritional importance are also reviewed in this section. In addition, literature related to physico-chemical and rheological characteristics of durum wheat and semolina in relation to their pasta-making quality are reviewed.

**Section III** or “**Materials and Methods**” includes details about the durum wheat varieties procured, chemicals obtained, instruments used and various methodologies employed in the study. Spectrophotometric determinations, colorimetric, Farinograph and Micro visco-amylograph studies, electrophoresis and HPLC techniques, scanning electron microscopy, and texture analysis studies along with other physico-chemical analysis carried out are all described with relevant literature references.

**Section IV** deals with the “**Results and Discussion**” of investigation work and is divided into 4 chapters, each containing a brief introduction. Results of each chapter are supported by suitable statistical analysis. Chapter 1 is divided into 2 sub-chapters 1A and 1B.

In **Chapter 1A**, 14 Indian durum wheat varieties procured from different Indian Agricultural Research Institutions and Universities, were analyzed for their proximate composition such as moisture, ash, protein, and yellow pigment content. In addition, activity of enzymes such as peroxidase (POD), polyphenol oxidase (PPO), lipoxygenase (LOX), and protease, which are important in ensuring quality of pasta, was determined. High molecular weight glutenin subunits (HMW-GS) of these varieties were identified by SDS-PAGE analysis. Results showed that the protein content of 14 Indian durum wheat varieties varied from 10.7% (WH 896) to 15.9% (MACS 1967), and their yellow pigment content ranged from 3.8 ppm (MACS 2694) to 7.2 ppm (DWR 2006). The optimum pH for POD, PPO, and LOX was found to be 5.0, 7.5, and 5.5, respectively. The POD activity

in these varieties varied from 269 U/g (PDW 233) to 1010 U/g (NIDW 15), and PPO activity from 53.8 U/g (PDW 215) to 78.3 U/g (MACS 1967). The PPO activity in these durum varieties was significantly lower than those of two Indian aestivum varieties tested. The LOX activity ranged between 1.4 U/g (MACS 2846) and 6.9 U/g (MACS 1967). The protease activity was in the range of 1.1-5.1 U/g, which belonged to varieties MACS 2694 and MACS 1967, respectively. Amongst the 14 durum varieties analyzed, four HMW-GS compositions including subunits 6+8, 7+8, 13+16, and 20 were identified in 3, 6, 3, and 2 varieties, respectively. Based on these initial studies, six Indian durum wheat varieties, namely, DWR 2006, MACS 1967, MACS 2694, PDW 215, PDW 274, and WH 896 were selected for further studies in Chapter 2.

In **Chapter 1B**, Buhler laboratory mill was standardized for semolina milling. Various roll gap spaces for break rolls B1 and B3, different tempering times and conditioning moisture levels were used to optimize the milling conditions for producing desired semolina. Results showed that gap spaces of 0.4 and 0.2 mm for break rolls B1 and B3, respectively, and tempering moisture of 17% for 18 h, were optimum conditions for producing a semolina with desired yield, particle size and ash content.

In **Chapter 2**, six Indian durum wheat varieties, namely, DWR 2006, MACS 1967, MACS 2694, PDW 215, PDW 274, and WH 896, which were selected based on initial studies in chapter 1A, were examined for their physical, chemical, biochemical, and semolina milling properties. Semolina

samples were evaluated for their physico-chemical, Farinograph, Amylograph, and spaghetti-making properties. Results showed significant correlations between some of the physical properties of wheat kernels and semolina milling yield. Semolina samples from different varieties showed significant differences in their physical characteristics such as color, scanning electron micrographs, Farinograph, and starch pasting properties. Semolina samples also showed significant differences in their protein, yellow pigment, wet gluten, and acetic acid insoluble protein contents. Significant correlations were found between total protein, wet gluten, and particle size distribution of semolina samples, with their Farinograph mixing properties. In addition, relationships existed between physico-chemical characteristics of semolina and color, and scanning electron micrographs of dry spaghetti samples. Relationships were also observed between physico-chemical characteristics of semolina samples and cooking quality properties of spaghetti such as cooking loss, cooked weight, firmness, and stickiness. Subjective and objective evaluations showed that MACS 1967 and PDW 274 were excellent and poor durum varieties, respectively, with respect to spaghetti production. Results of SDS-PAGE showed that a 45 kDa protein was absent in poor durum variety PDW 274. A peak designated as GliPK 36-37 was also absent in poor variety PDW 274. SDS-PAGE of albumins, globulins, gliadins, and glutenins fractions of good and poor varieties showed that there was no difference between their albumins and globulins fractions, but significant differences were observed between gliadins and glutenins.



In **Chapter 3**, effect of low temperature (LT) and high temperature (HT) drying processes on physico-chemical, microstructure, and cooking quality characteristics of spaghetti from three durum varieties, namely, DWR 2006, MACS 1967, and PDW 274, two good and one poor varieties, respectively, was investigated. Results showed significant improvement in yellow pigment retention and color characteristics of spaghetti processed by HT compared to LT-dried spaghetti. Scanning electron micrographs of spaghetti surface showed a smoother surface with a more continuous protein matrix due to HT drying process, indicative of significant improvement in microstructure of spaghetti. Starch pasting properties of mixed dough, extruded dough, and LT- and HT-dried spaghetti samples showed significant differences among three varieties and within each variety, during processing. HT drying process exhibited substantial effect on distribution and solubility of protein fractions compared to LT drying process. Cooking loss, firmness, and stickiness of HT-dried spaghetti samples from good and poor durum varieties significantly improved compared to corresponding LT-dried spaghetti. Effect of different cooking times (4-20 min) on cooking quality parameters, microstructure, and pasting properties indicated obvious differences between spaghetti from good and poor varieties, and also between spaghetti processed by HT and LT drying. Results were indicative of the effect of not only protein but also starch and starch-protein interactions on cooking quality of spaghetti.

In **Chapter 4**, effect of microbial transglutaminase (TG) and microbial lipase on semolina dough properties and spaghetti quality of good and poor

durum varieties MACS 1967 and PDW 274, respectively, was investigated. Results clearly demonstrated the effects of TG on the solubility and also on the SDS-PAGE patterns of durum wheat proteins. Protein cross-linking reaction catalyzed by TG resulted in changes in pasting properties of semolina, dough properties, dry spaghetti quality, cooking quality properties, and microstructure of cooked spaghetti. The quality improvements were more evident in spaghetti from low protein-poor variety PDW 274. The results also showed the ability of TG in formation of heterologous polymers between soya proteins and durum wheat proteins to improve the quality of spaghetti samples. Microbial lipase also improved the breaking strength of dry spaghetti and firmness and stickiness of cooked spaghetti samples.

**Section V** covers “**Summary and Conclusions**” of the investigation, which includes the salient findings of the work followed by general conclusions.

**Section VI** includes the collective bibliography (references) of citations quoted in all the chapters.

Wheat is among the oldest and most extensively grown of all crops (Orth and Shellenberger, 1988). Historic documents confirm that wheat is the earliest field crop used for human food processing (Posner, 2000). It also became the leading grain used for human consumption due to its nutritive profile and relatively easy harvesting, storing, transportation, and processing as compared to other grains (Posner, 2000). Nowadays wheat is made more than a food source and holds a place of importance in human affairs. It has become synonymous with sophisticated living and is a political and highly emotive commodity. It continues to be one of the world's most important grains, especially as a food, where the unique properties of its products can be utilized to advantage. It provides an excellent example of a natural product from which a wide range of useful by-products can be made (Cornell and Hoveling, 1998).

### **1.1. Classification of wheat**

Many wheat kinds and classes, available around the world, vary in quality as a result of climate, irrigation, specific variety characteristics, growing conditions, harvesting, and handling (Posner, 2000). All wild and cultivated wheats are members of the genus *Triticum*, and economically they are probably the most important group within the large grass family, the gramineae (Poaceae) and the Hordeae tribe (Wiseman, 2001). Linneus suggested the first classification of wheat in 1753. His classification was based upon physiological and morphological differences between the wheats. However, since Linneus there have been a number of proposed classifications of the genus *triticum* (Wiseman, 2001). The cytogenetic and

cytologic work of scientists showed that wheats fall into three basic natural groups, each one characterized by having 14 chromosomes (as seven pairs) or a multiple of 14 chromosomes in each somatic cell (Bozzini, 1988). The simplest group, genetically at least, are the diploid wheats that have two sets of seven chromosomes (14 chromosomes in total); this group has *T. monococcum* (einkorn wheat) as a member. The chromosomes present in these species are categorized into a genome (designated A in *T. monococcum*). The second group is the tetraploid wheats, two important members being *T. durum* (durum or macaroni wheat) and *T. dicoccum*. The tetraploid wheats have four sets of seven chromosomes (28 chromosomes in total). In this case the four sets of chromosomes are categorized into two genomes (designated A and B in *T. durum*). The third group, containing amongst others, the economically important bread wheats (*T. aestivum*), are the hexaploid wheats. Each member has six sets of seven chromosomes (42 chromosomes in total), the chromosomes being categorized into three genomes (designated A, B and D in *T. aestivum*).

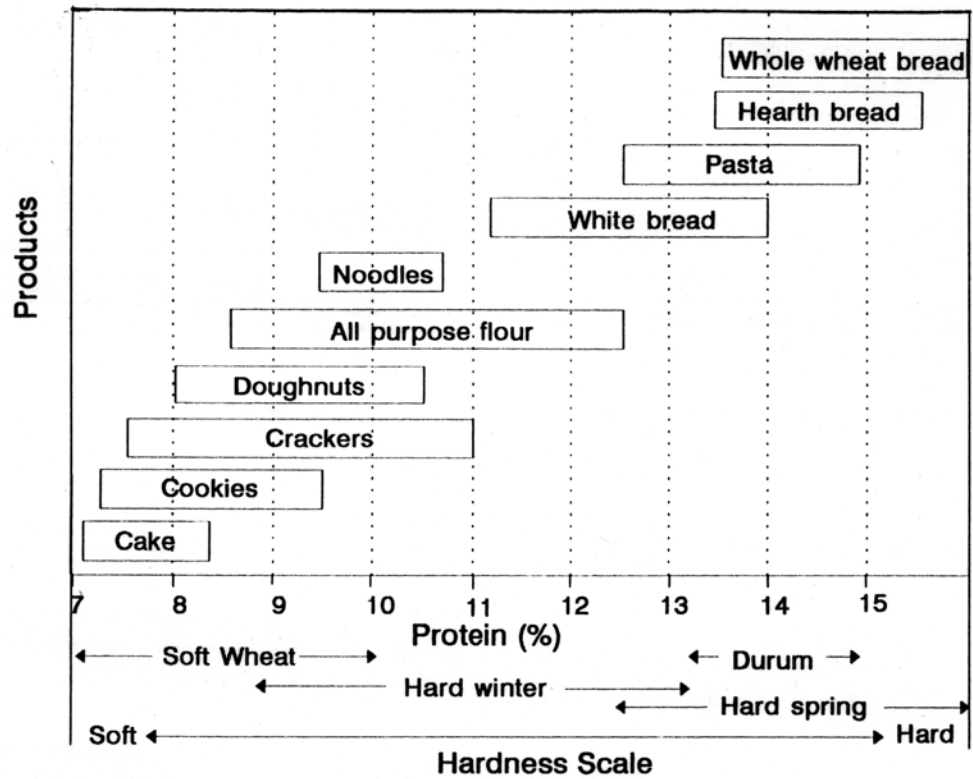
Genetic and cytogenetic analyses conducted mainly at the hexaploid level have demonstrated that the chromosomes of the three basic genomes in hexaploids (ABD) or two basic genomes in tetraploids (AB) can be grouped into seven basic types (Bozzini, 1988):

1A	2A	3A	4A	5A	6A	7A	<b>2n</b>	}	<b>4n</b>
1B	2B	3B	4B	5B	6B	7B			
1D	2D	3D	4D	5D	6D	7D		}	<b>6n</b>

For commercial purposes, a system of classification based upon physical and chemical properties is used. The major factors used to distinguish wheats are hardness or softness of the grain, winter or spring habit, red or white bran, and protein content (Orth and Shellenberger, 1988). For example, soft, hard, or durum wheat differ in endosperm structure, hardness, and protein characteristics. Consequently, they are milled differently and the resulting milled products are suitable for different end uses, i.e. durum wheats are preferred for pasta, soft wheats for biscuits, cakes, and pastries, and hard wheats for bread, noodles, flat breads, pan bread and other products. Combination of high protein and hard kernels results in flour most suited for products such as pan bread, and foods made from durum wheat semolina. Fig.1 shows a schematic diagram of the relationship between protein percentage, wheat type, hardness, and end-product utilization (Posner, 2000).

## **1.2. Wheat: World and Indian scenario**

Most of the wheat varieties cultivated today are grouped together under the broad category of common or bread wheat, which accounts for approximately 95% of world production. Nearly all of the remaining 5% of cultivated varieties are durum wheats used for such products as pasta and couscous. Wheat has the widest adaptation of all cereal crops and is grown in some 100 countries around the world. It is grown as far north as Finland and as far south as Argentina. The heaviest concentration is in the temperate zone of the northern hemisphere between the 30<sup>th</sup> and 60<sup>th</sup> latitudes, which includes the major grain growing areas of North America, Europe, Asia and



**Fig. 1.** Schematic diagram of relationship between percentages of protein, wheat type, hardness, and end-product utilization (Posner, 2000).

North Africa. There is also some lesser concentration between the 27<sup>th</sup> and 40<sup>th</sup> latitudes in the south, chiefly Australia, Argentina, Brazil and South Africa (Oleson, 1994).

Looking at the last seven years prior to 2005-06, total world wheat production has averaged to 578 million tones (Mt) from 589.7 Mt in 1998-99 to 598 Mt in 2004-05 (Lennox, 2002, 2003b; Lennox and Morgan, 2005). Wheat production is highly concentrated so that just three producers, the former Soviet Union, China and the European Union, account for almost half of the world production, producing 72.6 Mt, 91.3 Mt and 136.4 Mt, respectively. When the USA (58 Mt), India (72 Mt) and Canada (20.8 Mt) are added, the top six producers account for about more than three quarters of world wheat production. Nine major wheat producers in the world for last seven years are shown in Table 1.

USA, European Union, Canada, Australia, Argentina and former Soviet Union are six major exporters in the world. They have exported 87.3, 82.3, 94.7 and 97.6 Mt wheat in 2002, 2003, 2004 and 2005 (forecast) respectively. Fig. 2 shows the wheat exports by these countries (Lennox, 2003b; Lennox and Morgan, 2005). India, with an annual wheat production of 72.0 Mt, is the second largest wheat producing country in the world (next only to China) and one of the leading examples of the 'green revolution'. Indian wheat production has been steadily increasing, resulting in a sharp build up of stock. Production increased to about six-fold in the last two and a half decades and reached a record 76.4 Mt in 2000-2001. Consequently, India has been

**Table 1.** World production of wheat (million tones) (Lennox, 2002, 2003b; Lennox and Morgan, 2005).

	1998-99	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06 <sup>A</sup>
<b>EU-15</b>	103.1	96.4	104.8	91.7	104.4	92.5	136.4 <sup>B</sup>	125.5 <sup>B</sup>
<b>China</b>	109.7	113.9	99.6	93.9	90.6	87.0	91.3	95.0
<b>Former SU</b>	57.6	66.1	64.8	92.9	99.2	64.0	72.6	77.0
<b>India</b>	66.4	70.8	76.4	68.8	71.8	67.0	72.0	72.0
<b>US</b>	69.3	62.6	60.8	53.3	43.9	62.4	58.0	59.0
<b>Eastern Europe</b>	33.9	28.2	28.7	35.2	30.5	22.0	-	-
<b>Australia</b>	21.5	24.8	23.8	24.0	9.4	24.0	21.5	21.5
<b>Canada</b>	24.1	26.9	26.8	20.6	12.0	17.2	20.8	19.6 <sup>C</sup>
<b>Argentina</b>	13.3	16.4	16.2	15.5	12.3	13.5	16.6	13.5
<b>Other</b>	90.8	80.1	82.0	82.9	89.9	97.4	108	127
<b>World</b>	<b>589.7</b>	<b>586.2</b>	<b>583.9</b>	<b>578.8</b>	<b>564.0</b>	<b>547.0</b>	<b>598.0</b>	<b>610.0</b>

A, Forecasted by USDA

B, EU-25

C, Forecasted by Statistics Canada (SC)



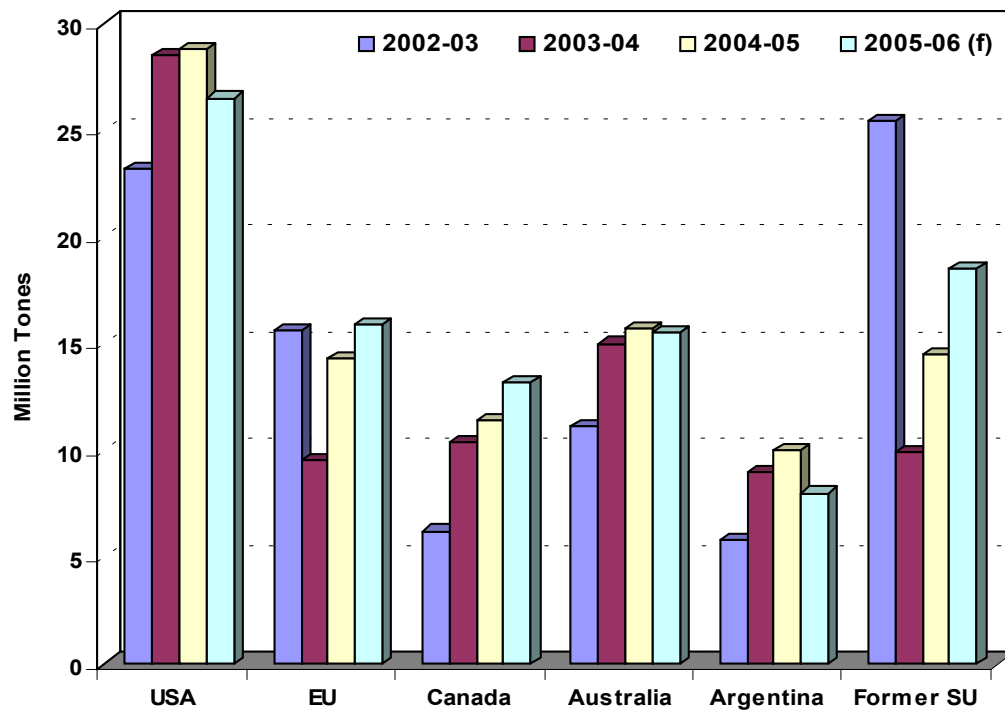
transformed from a major wheat importer to an occasional exporter. Therefore, in spite of population increase, the per capita availability of wheat has increased from 42.5 to 65.0 Kg per annum in the last 25 years. In view of the surplus position of wheat and its export potential, it has become pertinent for India to focus on quality (Srivastava, 2000). Indian wheat exports were 5.0 Mt and 5.7 Mt in 2002-03 and 2003-04 respectively, although much has been exported as feed into Southeast Asia (Lennox, 2003b; Lennox and Morgan, 2005). Wheat price is one of the main constraints on India's wheat exports. Indian market price is around US\$ 150 per tone whereas the international price is between US\$ 120-130 ([www.indiaonestop.com/wheat.htm](http://www.indiaonestop.com/wheat.htm)).

### **1.3. Durum Wheat**

#### **1.3.1. Origin**

Radio carbon dating has been used to establish that species of wheat were being cultivated around 8400-7500 B.C. in areas that are now part of Iraq, and Syria. Evidence suggests that durum wheat was grown by the Egyptians around 4000 B.C. It has been also speculated that durum was grown around the same period in the area that is now the Ukraine (Wiseman, 2001).

It is probably safe to state that durum wheat originated in the Middle East, where it is still produced and consumed in substantial quantities. Durum wheat was introduced to the New World in 1527, when it was brought to Argentina, but did not become an important crop until the beginning of the present century (Dick and Matsuo, 1988).

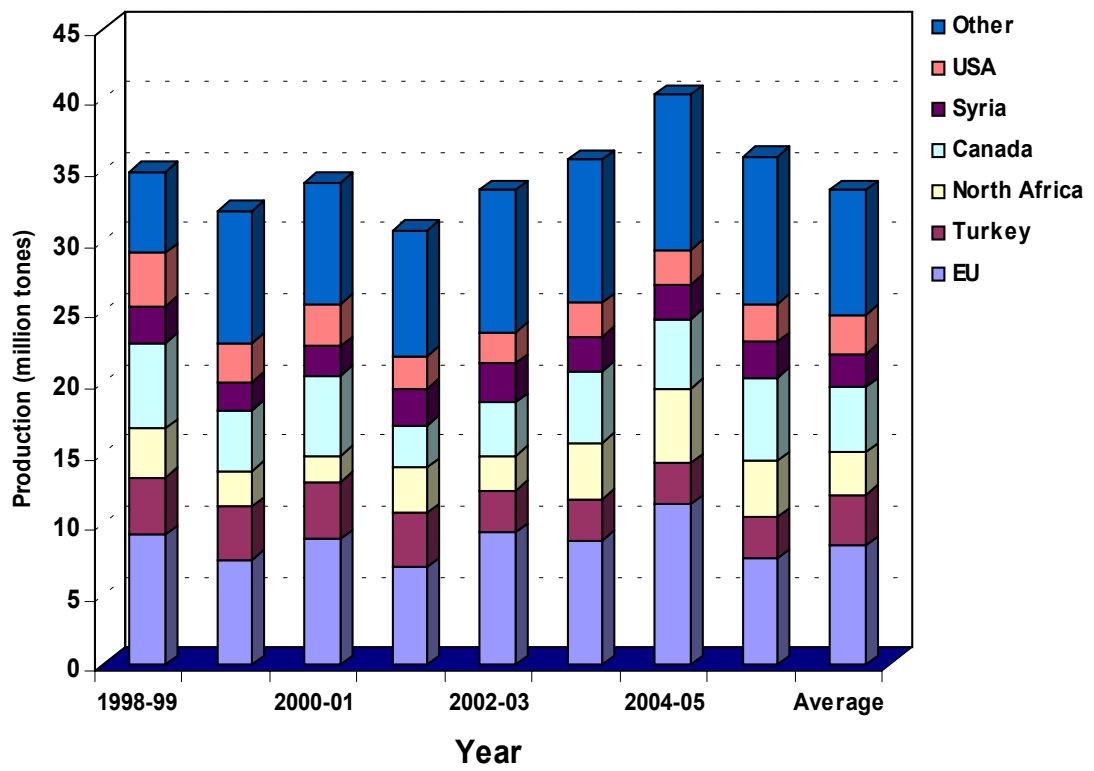


**Fig. 2.** Major world wheat exporters (f: forecasted) (Lennox, 2003b; Lennox and Morgan, 2005).

### 1.3.2. World production

Durum wheat is grown only in certain parts of the world unlike common wheat which can be grown much more widely. The best quality durum is produced in regions having a relatively dry climate, with hot days and cool nights, during the growing season. Durum produced under conditions of higher moisture tends to have a low count of hard vitreous kernel, making it less suitable for the production of pasta. Traditional durum consumption therefore developed in the hot dry regions around the Mediterranean such as North Africa, southern Europe, Turkey and Syria.

World durum production for 2005-2006 is estimated by International Grains Council (IGC) at 35.9 Mt, an 11 percent decrease from 2004-2005 (Lennox, 2005). Accordingly, looking at the seven years prior to 2005-06, total world durum wheat production has averaged to 34.6 Mt from 34.8 Mt in 1998-99 to 35.9 Mt in 2005-06, contributing to around 6.0 percent of the average of total world wheat production in the same period. Production has been concentrated in the twenty-five member countries of the European Union (25%), the Middle East, largely Turkey and Syria (18%), North Africa (9%), Canada (14%) and the United States (8%). Durum wheat production in five major durum wheat producers and world durum wheat production is shown in Fig. 3 (Lennox, 2001, 2003a; 2005; Connel et al, 2004). Other durum wheat producers such as Kazakhstan, India, Mexico and Australia can be put in same order after Syria. Their durum wheat production in 2002-2003 was 2.4, 2.1, 1.1 and 0.3 Mt, respectively (Lennox, 2003a).

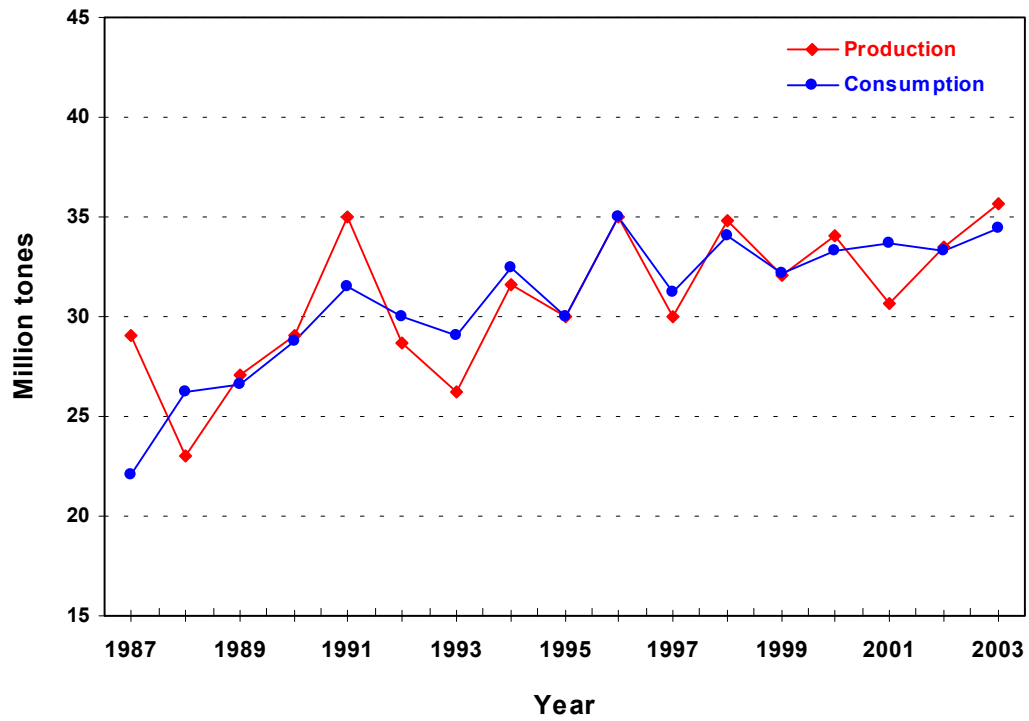


**Fig. 3.** Durum wheat production in five major durum wheat producers and world durum wheat production (Lennox, 2001, 2003a, 2005; according to IGC data).

Durum wheat use is largely concentrated in the European Union, particularly Italy, followed by North Africa and the Middle East (Connel et al, 2004). European and American countries almost totally use durum wheat for pasta products, whereas in the Middle East and North Africa, local bread making accounts for about half of the consumption and about equal shares are used for pasta, couscous (small balls of semolina steamed and prepared in a similar manner to rice), *burghul* and *frekeh* or consumption as feed (Bozzini, 1988). Durum wheat is also used in the preparation of two-layered breads, known by various names in different countries such as, *khobz*, *baladi* and *shami*; single-layered breads, such as *tannour*, *saaj*, mountain bread, *markouk* and *mehrahrak*. Burghul (or sometimes spelled bulgur, boughur, borghol, boughour) is a granulated, boiled, sun-dried durum wheat used in several ways, ranging from steamed burghul much like rice, to burghul/minced meat dishes and sweets. Another durum-based product, frekeh, is green, dried, parched wheat, ground into coarse particle size and steamed like rice. Therefore, although durum wheat as a class might be considered a minor crop compared to common wheat, the diets of millions of people in the Middle East and North Africa are based on durum wheat (Matsuo, 1994). World durum wheat production and consumption during 1987 to 2003 is shown in Fig. 4 (Lennox, 2001, 2003a).

### 1.3.3. Durum wheat exports and imports

According to data from IGC, in the three consecutive years prior to 2001-02, Canada was the major exporter, shipping 50 percent of all exports, followed by the United States (19%), the European Union (8%), Mexico (7%),



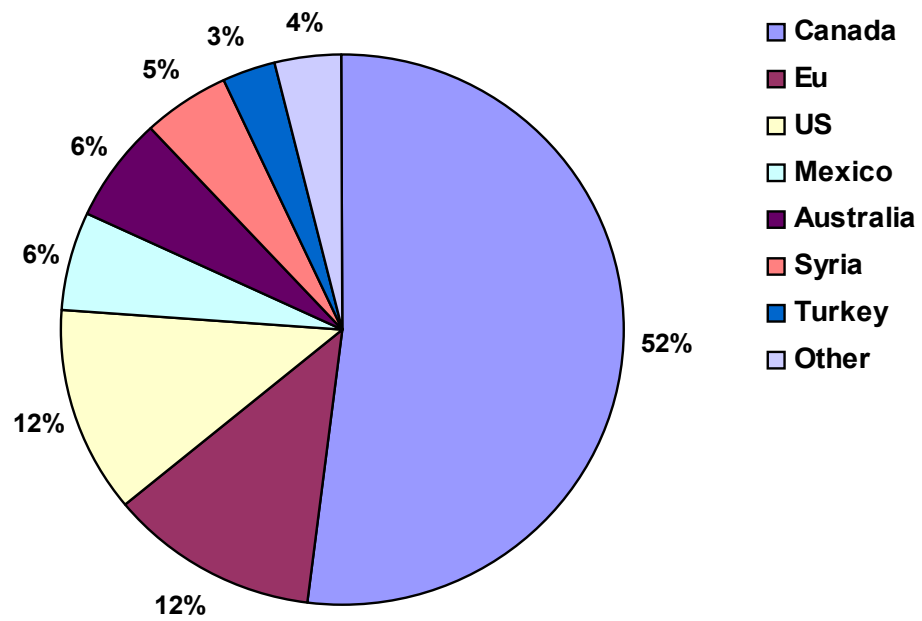
**Fig. 4.** World durum wheat production and consumption during 1987 – 2003 (Lennox, 2001; 2003a).

Turkey (6%) and Australia (5%), respectively. The export reported for 2003-2004 by the IGC put Canada's share at 52 percent, while that of US and EU at 12 percent each. According to IGC estimation, Canada shared 45% and 47% of the world durum market in 2004-05 and 2005-06, respectively, which was below the 10-year average of 50% (Lennox, 2005). Although EU is the largest producer, it is also a high net consumer of durum wheat (Fig. 5).

In the three consecutive years prior to 2003-04, North African countries imported around 44 percent of world imports which make them the largest durum import market. The other major regional market is the EU, taking 23 per cent of world imports. Italy has been the major durum wheat importer of the European Union. Other markets have included the US (7%), Venezuela (4%) and Japan (3%) (Connel et al., 2004). Fig. 6 shows the average of durum wheat imports for years 2000 and 2001.

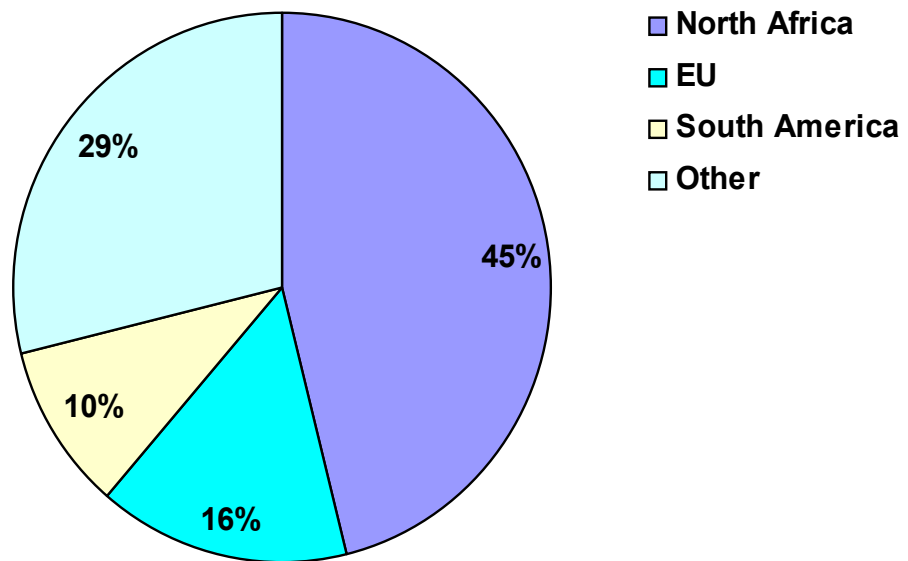
#### **1.3.4. Durum Wheat: Indian Scenario**

Wheat production in India has significantly increased during the several decades as a result of the "green revolution". Along with that, Indian durum wheat production has also been gradually rising upward, from about 1.0 Mt in the late 1980s to 2.5 Mt in 2003. Presently, cultivation of durum wheat in India is spread out in pockets of central India, including Madhya Pradesh and the state of Punjab. Indian durum varieties have a high level of resistance to leaf rust and diseases like loose smut and karnal bunt. Therefore, farmers in India want to grow more durum wheat than bread wheat (Kathuria and Sidhu, 1984a).



**Fig. 5.** World durum wheat export for 2003-04  
(Lennox, 2003a; according to IGC data).





**Fig. 6.** Major durum wheat importers (Lennox, 2001; average of 2000 and 2001 data of IGC).

Even though India has the potential to export substantial quantities of durum wheat to the world market, especially Middle-Eastern countries, South Africa and Mediterranean countries, majority of the 2.5 Mt of durum wheat produced by Indian farmers is consumed within the domestic market for preparation of several Indian traditional dishes. For example, durum semolina colloquially known as '*Bansi*' along with farina is marketed for use in the preparation of '*upma*', '*kesaribath*' (shira), '*ladu*', '*semia*' (vermicelli) etc. Roasting of semolina is a popular practice in many households for preparation of semolina based food items (Ranga Rao et al., 1981). However, it needs mentioning that previously much of Indian durum wheat has been rejected in the international market for failing to meet the standards of purity especially admixture with bread wheat.

### **Scope and Objectives**

Durum wheat is the most suitable raw material for the production of pasta products. It is estimated that around 50 percent of world durum wheat production is converted into pasta products such as spaghetti, macaroni, vermicelli etc. India is not only the second largest wheat producer in the world but also a major durum wheat producer. In recent years, different Indian Agricultural Research Institutions and stations have developed several new durum wheat varieties. In addition, pasta products are also becoming more and more popular within the Indian consumers. Therefore, though India has the potential to export its durum wheat to the world market, the domestic market for durum wheat is also expanding. The rapid increase in demand for Indian durum wheat by domestic market has not been accompanied by in-

depth sufficient research work in this area, and there is little information available regarding the characteristics of Indian durum wheat and its semolina milling quality in relation to their suitability for pasta, especially spaghetti production. This research work was carried out to overcome a part of this lacuna and was based on three major objectives:

- 1-** To study the physico-chemical properties of Indian durum wheat
- 2-** To study the semolina milling quality of Indian durum wheat
- 3-** To study the spaghetti-making quality of Indian durum wheat

## 2.1. Durum wheat

Durum wheat (*Triticum durum*) is a tetraploid wheat and a separate species from most other commercially grown wheat classes (which are mainly *T. aestivum*, a hexaploid wheat) and it has unique characteristics (Lennox, 2003a). Durum wheat is generally regarded as being a larger grain with a more elongated shape than the more common *T. aestivum*, which is usually smaller and more oval in shape (Wiseman, 2001). Durum, from Latin word for 'hard' is an appropriate name for the firmest of all wheats. Its hard, tough and horny endosperm facilitates a yield of good quality semolina that is higher than that from *aestivum* wheats (Bolling and Zwingelberg, 1974, 1975). The inherent hardness of durum wheat is possibly related to the absence of a specific protein on the surface of the starch granules (Greenwell and Schofield, 1986).

Durum wheats generally have higher protein contents, higher levels of carotenoid pigments, lower lipoxygenase activity, and better resistance to some wheat diseases (Kathuria and Sidhu, 1984a). Good quality durum has a very hard vitreous (i.e. glassy looking) kernel, with an amber yellow endosperm, compared to the white endosperm of common wheat. Pasta made from durum semolina maintains a desirable firm texture during cooking, and it has a natural amber color that is associated with good quality pasta (Lennox, 2003a). Therefore, durum wheat is considered the raw material of choice for the production of pasta products (Dick and Matsuo, 1988). Feillet and Dexter (1998) consider durum as the preferred wheat for pasta production due to its unique color, flavor, and cooking quality.

## 2.2. Quality aspects of durum wheat for milling

Durum wheat milling is different from other forms of wheat milling, in that, there are differences in the wheat, the actual milling process, final product characteristics and the utilization of those final products (Robinson, 2001). The endosperm, or heart of the wheat kernel, does not breakdown into a fine powdery flour when milled, because the endosperm of durum is hard enough to hold together during milling, and the result is a granular product called *semolina*, which is used to make spaghetti and other pasta products (Connell et al., 2004). The term 'semolina' is derived from the Italian word '*semola*' and the French equivalent '*semoule*' and is defined as the purified middlings of durum wheat, which has been ground so that all of the products pass through a No. 22 U.S. sieve and not more than 3% shall pass through a No. 100 U.S. sieve (Donnelly and Ponte, 2000).

Some of the parameters for wheat selection for semolina milling include test weight, kernel size, degree of vitreousness, protein content, and ash content (Robinson, 2001). Test weight is a reflection of the soundness of grain, and thus grain undamaged by environmental stresses has a high test weight. In general, test weight is related to semolina yield (Matsuo, 1988). Durum wheat test weight is a good predictor of semolina milling potential because it exhibits a strong linear relationship to kernel weight (Dexter et al., 1987, 1991).

The 1000-kernel weight is a measure of average kernel size. A greater milling yield is expected with larger kernels because the ratio of endosperm to

bran should be greater (Matsuo, 1988). In reality, no valid study demonstrates that all varieties of durum wheat with small kernels, and therefore with low 1000-kernel weight, have a potentially lower capacity to produce high semolina yield (Cubadda, 1988). Matsuo and Dexter (1980b) reported that milling yield was affected only when the kernels fell below a certain size. Kernel weight is the best single index of potential semolina yield and should be considered an important factor in assessing durum wheat quality (Dick and Matsuo, 1988). Vitreousness is also an important factor in milling. Non vitreous kernels are those that are starchy, partly starchy, severely damaged, broken, or from wheats of other classes (Matsuo, 1988). Matsuo and Dexter (1980b) indicated that as starchy kernel content increased, milling yield was not affected but the proportion of flour increased. Dexter and Matsuo (1981) showed that as starchy kernel content increased, semolina granulation became finer and more flour was produced during milling. Dexter et al. (1988, 1989) found that fully starchy durum wheat kernels were softer than vitreous kernels, but partially vitreous kernels were comparable in hardness to fully vitreous kernels.

Although the previous factors are important for evaluation of durum wheat for semolina milling, several European milling industries consider ash content as the most important aspect of durum wheat quality for semolina milling (Troccoli et al., 2000). Experimental results have confirmed a significant role of genotype-environment interactions in determining ash content (Fares et al., 1995; Peterson et al., 1986). With higher ash content in whole grain, a lower semolina yield can be expected because the ash content

of semolina is correlated with that of the whole kernel (Cubadda, 1988) and with the extraction rate (Dexter and Matsuo, 1978a). Furthermore, this parameter is especially important in several European countries where the ash content of durum wheat products for human consumption is regulated by law. For example, in Italy ash content must not exceed 0.9% (dry matter basis) for first grade commercial semolina (Troccoli et al., 2000).

Grain Moisture content represents another aspect of quality for durum wheat milling. The percentages of other components, such as protein and starch and consequently kernel test weight are inversely related to moisture content. In addition, grain in excess of 13.5% moisture may heat during storage, with sprouting, fungal damage and deterioration in condition (Troccoli et al., 2000).

### **2.3. Semolina milling and quality aspects of semolina**

The process of milling durum varies, depending on the desired characteristics of the mill products and whether the operation is for experimental or for commercial reasons. The objective of experimental durum milling is to produce a milled product of adequate quality to evaluate the potential value of wheat or milling technique (Dick and Matsuo, 1988). Because the durum kernel is very hard, it yields when subjected to grinding, relatively high amounts of large particles (semolina) (Miller et al., 1982). As mentioned in the previous section (2.2), semolina is a granular product, which is analogous to the *farina* or flour middlings of the common hard wheat milling process (Bass, 1988).

Economically, it is advantageous to maximize the yield of semolina and minimize flour production since semolina commands a higher selling price. A good commercial mill can be expected to obtain extractions of 60-64% semolina and 8-12% flour (Banasik, 1981). However, according to Feillet and Dexter (1998), the semolina extraction of a modern, well-equipped mill solely dedicated to durum wheat milling range from 65-75%. Dick and Youngs (1988) stated that milling performance of durum wheat is evaluated by semolina extraction, total extraction (semolina+ flour), and appearance and granulation of the semolina. Durum wheat milling has been reviewed in detail by Bizzarri and Morelli (1988). Milling tests performed on experimental equipment are useful for comparing different varieties and for obtaining comparison data among different samples rather than absolute values (Cubadda, 1988). Black and Bushuk (1967) and Sollberger (1970) have described how the Buhler laboratory mill can be used to produce semolina of good quality. Dexter et al (1982) described a method for milling durum wheat in the Buhler laboratory mill to yield semolina of comparable extraction and quality to commercial semolina. The very hard durum grain must be tempered to relatively high moisture content (16.0-16.5%) before grinding (Dick and Matsuo, 1988). This moisture content toughens the seed coat so that efficient separation of bran and endosperm can take place (Donnelly and Ponte, 2000). However, Bizzarri and Morelli (1988) reported that, for amber durum wheat, 16-24 h of tempering at 17-17.5% moisture and for white durum wheat, 12-16 h at 16-16.5% moisture provide typical conditions for milling.



Final steps of semolina milling involve purifying to separate as much of the small bran particles and flour from the semolina (Donnelly and Ponte, 2000). Good purification is required since bran or other dark particles are readily visible in the yellow semolina, and these dark particles or specks are perceived by the consumer as contaminants of pasta product (Dick and Matsuo, 1988). A development in durum wheat milling is preprocessing prior to milling, i.e. removal of bran layers by a series of modified rice polishing machines (McGee, 1992; Dexter et al., 1994). About 65% of the bran can be removed during this process (Feillet and Dexter, 1998).

Semolina quality requirements vary from country to country (Cubadda, 1988) and also pasta manufacturers have unique requirements for semolina (Dick and Matsuo, 1988). Several common factors such as moisture content, granulation, color, speck count, ash content, protein content and amylase activity are often considered when judging semolina quality.

Granulation or particle size distribution of semolina is important since it has an effect on the absorption properties of the pasta dough and therefore influences the quality of the finished pasta. The basic principle is that more even the particle size more even the hydration of the semolina in the pasta mixer. Traditionally the preference was for coarse semolina but now modern pasta making techniques require finer particles that hydrate quicker (Turnbull, 2001a). The finer durum semolina results in quicker and better water absorption and, therefore, in a shorter mixing time giving a homogenous dough. It has been determined that a semolina with particle size of 125 – 600

$\mu\text{m}$  requires a mixing time of 15 min for absorption, whereas for semolina of particle size below 350  $\mu\text{m}$  and 250  $\mu\text{m}$ , a mixing time of 10 and 5 min, respectively will be sufficient. From the industrial point of view, these semolinas are easier to process; enable translucent and homogenous end products (Kuenzli, 2001). If semolina is not uniform, but consists of fine as well as coarse particles, the fine particles will tend to absorb water faster than the larger particles, resulting in white specks in the pasta (Donnelly and Ponte, 2000). The rate of granulation of semolina differs from country to country. Table 2 shows particle size distribution for typical commercial semolina in USA, Canada, and Italy and also semolina milled at the grain research laboratory (GRL), Canada.

Specks in semolina can be divided into two types. Black and dominant specks are the result of inadequate wheat cleaning. Brown specks are smaller and less visible and predominantly the result of poor mill performance and flow design (Turnbull, 2001a). Semolina with fewer than 50 specks per 64.5  $\text{cm}^2$  (10  $\text{in}^2$ ) is desirable and gives pasta with a relatively nice appearance (Dick and Youngs, 1988).

The moisture content in semolina is an important factor in processing it into pasta. The final moisture content of semolina results from three different influences. First is the need to maximize mill gain by incorporating as much water as possible. Second is the ideal moisture content for optimal milling performance and product handling. Third is the overriding safety requirement not to exceed a moisture level where mould growth or other microbiological

**Table 2.** Particle size distribution for typical USA<sup>a</sup>, Canadian<sup>b</sup>, and Italian<sup>c</sup> commercial semolina and GRL<sup>b</sup> semolina

U.S. sieve no.	U.S. sieve opening (mm)	U.S. total semolina (%)	Canadian total semolina (%)	GRL total semolina (%)	Italian sieve opening ( $\mu$ )	Italian total semolina (%)
<b>On 20</b>	0.86	0.0	0.0	0.0	-	-
<b>On 40</b>	0.38	22.8	20.1	7.8	$\geq 315$	9
<b>On 60</b>	0.23	51.6	62.2	69.5	315-250	30
<b>On 80</b>	0.18	14.6	15.1	19.5	250	26
<b>On 100</b>	0.14	9.3	1.7	1.9	250-150	20
<b>Through100</b>	-	1.7	0.4	0.9	<150	15

<sup>a</sup> Banasik, 1981

<sup>b</sup> Matsuo, 1988

<sup>c</sup> Milatovic, 1991

problems could occur (Turnbull, 2001a). In other word, semolina moisture should be as high as is possible without risking the hazards of spoilage or deterioration during storage. An acceptable level is 13.5-14.5%. Too low a semolina moisture level can cause difficulties with hydration of liquid ingredients in the mixing step of pasta production (Dick and Matsuo, 1988).

Ash content is another important factor in the assessment of semolina quality. High ash content can produce a duller semolina color because of the presence of high-ash outer-endosperm particles (Cubadda, 1988). Detailed studies have shown that the least refined streams produce a product that is browner and duller (Matsuo and Dexter, 1980a). Therefore, semolina extraction rate is negatively correlated to spaghetti brightness (Dexter and Matsuo, 1978a). The ash content of endosperm taken from the centre of the grain can be as low as 0.6%, compared with durum flour taken from the area of the grain adjoining the aleurone layer of the bran skin which can be as high as 1.5% (Turnbull, 2001a). Typically, good quality pasta will be made from durum semolina with an ash level of less than 0.9% dwb. Pasta made from semolina with ash content between 0.9% and 1.1% dwb will be darker in color and its taste will be more 'wheaty'. Product manufactured from semolina with ash content above 1.1% will be very dark with a very strong flavor and, due to the high level of durum flour incorporated into such products, will have a poor texture (Turnbull, 2001b). Although ash content in the endosperm of durum is characteristically higher than in the endosperm of other hard wheats, the percentage of ash in durum semolina or flour can be still used as a relative measure of bran contamination (Dick and Matsuo, 1988).

The desired color of semolina is a clear bright yellow imparted mainly by xanthophylls, a carotenoid pigment (Irvine, 1971). Traditionally, a yellow color in semolina has been associated with good quality (Dick and Matsuo, 1988). Still, a yellow pasta is considered a mark of quality by many consumers because they assume that the product is made from durum wheat, which generally gives pasta with superior cooking quality compared to that made with non durum wheat (Dexter et al., 1981). Semolina color is useful for predicting pasta color and therefore is commonly measured for judging the color potential of durum samples (Dick and Youngs, 1988). However, the perceived color of semolina is related to the shape of the semolina particles, and the way in which light is reflected from them, as well as the inherent color of the wheat endosperm. It is therefore possible to have two semolina samples of different particle size that look different in color, but when converted to pasta is very similar in color (Turnbull, 2001a).

The protein content of semolina is an important guide to its quality. Generally, the higher the protein level the stronger will be the pasta, giving a better texture and less leaching of starch during cooking. However, there are numerous exceptions to this rule and this parameter can only be used as part of the 'total quality picture' of semolina (Turnbull, 2001b). Apart from protein quantity, protein quality also plays an important role in determining the semolina and pasta quality. This parameter will be discussed later in more detail.

High levels of  $\alpha$ -amylase enzyme in semolina due to sprout damage, influence the quality of the finished pasta. Although the color of pasta does

not appear to be appreciably affected (Dick et al., 1974; Donnelly, 1980; Matsuo, Dexter, and MacGregor, 1982), the mechanical strength of dry pasta seems to be weakened (Donnelly, 1980) and pasta cooking quality slightly affected by high amylolytic activity (Matsuo, Dexter, and MacGregor, 1982; Dexter et al., 1990). Maier (1980) showed that sprout damage does not however, appear to be as detrimental to spaghetti quality as some have claimed. Falling number test is the most widely used method for measurement of  $\alpha$ -amylase activity (Dick and Matsuo, 1988). Many American and Japanese manufacturers demand a durum wheat falling number in excess of 300 seconds (Dexter et al., 1990). The falling number near, equal to, or greater than 300 seconds indicates a weak or very weak  $\alpha$ -amylase activity, between 200 and 250 indicates normal activity; between 150 and 200, or less than 150 indicates high or very high  $\alpha$ -amylase activity (Mondelli, 1999). Falling numbers below 250 seconds are known to give problems in pasta production (Turnbull, 2001b).

#### **2.4. Durum wheat products**

Foods made from durum wheat can be grouped into the two general categories of 'Paste' and 'Non paste' products. Paste products are produced by mixing water and either durum semolina, durum flour, or whole ground durum to make an unleavened dough and then forming the dough into various shapes of the desired dimensions. These are consumed fresh or processed further to preserve the product. Examples of paste products are pasta and couscous (Dick and Matsuo, 1988). The non paste products are those foods

that either utilize the milled products of durum in a high-moisture dough (e.g. leavened or unleavened bread) or are the results of a cooking, steaming, or scorching process performed on the whole kernel of durum such as bulgur or frekeh (Dick and Matsuo, 1988).

#### **2.4.1. Pasta**

Pasta is the most commonly consumed paste-product made from durum wheat. The term 'pasta' has generally been reserved to describe paste products fitting the Italian style of extruded foods such as spaghetti or lasagna, and is usually distinguished from the Oriental style of sheeted and cut foods called 'noodles', which are commonly made from wheat other than durum (Dick and Matsuo, 1988).

Italians, who are the largest consumers of pasta products in the world, call these products '*pasta alimentare*' (alimentary paste) (Dick and Matsuo, 1988; Donnelly and Ponte, 2000). Pasta is a traditional food product with origins dating back to the first century B.C. (Agnesi, 1998) which is becoming increasingly popular world wide because of its convenience, nutritional quality and palatability (Cubadda, 1994). Pasta can be categorized into four main groups or types: (i) long-goods such as spaghetti, vermicelli, and linguine; (ii) short-goods include elbow macaroni, rigatoni, and ziti; (iii) egg noodles consists of pasta made with egg; (iv) specialty items such as lasagna, manicotti, jumbo shells, and stuffed pasta. Over 600 pasta shapes are produced, however, the number of sizes and shapes that can be produced is virtually unlimited and depends on the shape of the die from which the product

is extruded or the cutter with which it is cut. Spaghetti which is in the form of solid rods, elbow macaroni, lasagna, shells, and various noodle shapes are among the most popular shapes (Dick and Matsuo, 1988).

Although pasta is traditionally manufactured using only durum wheat semolina, pasta is sometimes also made from non-durum wheat flour, or farina or mixtures of durum and common wheat because common wheat is traded at a lower price than durum wheat. Several European countries including Italy, France, Spain and Greece take the view that the incorporation of any common wheat in pasta is effectively adulteration. Therefore a number of different biochemical and molecular techniques have been devised to address the problem of detecting the presence of common wheat in pasta products (Barnwell et al., 1994; Feillet et al., 1996; Wiseman, 2001).

In recent years pasta has become more popular due to its nutritional properties, being regarded as a product with low glycemic index (Jenkins et al., 1988; Wolever, 1990; Björk et al., 2000). The nutritional value of pasta derives from its high energy value (around 350 kcal per 100 g), reasonable protein content (11-12%) and its digestibility; its mineral content is unbalanced with a marked prevalence of potassium (Ferrari and Piazza, 2006). Pasta also provides significant quantities of complex carbohydrates, protein, B-vitamins, and iron and is low in sodium and total fat (Douglass and Matthews, 1982). Durum gliadin proteins are not toxic or are much less toxic to gluten-intolerant individuals than are gliadins from hexaploid wheats (Auricchio et al., 1985; Costantini, 1985). Normally when we eat pasta, it is accompanied by a series



of adjuncts or sauces such as vegetables (source of vit. A and C), fish, meat and eggs (source of protein), and/or legumes (source of protein and fiber) that enhance and improve its nutritional value through a sort of complementary process (Ferrari and Piazza, 2006). Table 3 shows the average composition of 100 g pasta.

#### **2.4.2. Bread making properties of durum wheat**

Although durum is not usually thought of as bread wheat, its use for bread is quite substantial in the Near East, Middle East, and Italy and occurs in lesser amounts in other countries (Williams et al., 1984; Williams, 1985). As much as 50% durum production is used for local breads in the Middle East and North Africa (Bozzini, 1988).

Generally, durum wheat is considered unsuitable for commercial scale breadmaking operations because it lacks the gluten strength found in most bread wheats (Boyacioglu and D'Appolonia, 1994). However, improvement might be possible through breeding, as suggested by studies conducted by Dexter et al (1981); Boggini et al (1988); Boggini and Pogna (1989) and Peña et al (1994). For durum wheats to achieve baking performances comparable to those of bread wheats, substantial improvements in gluten strength and most importantly, gluten extensibility are required (Ammar et al., 2000). The stronger durums, when blended with weak and soft wheats were reported to give flour mixes with improved bread making quality (Prabhavathi et al., 1976; Liu et al., 1996).

**Table 3.** Average composition of dry pasta

<b>Composition</b>	<b>Content (per 100 g)</b>
Moisture (g)	12.4
Proteins (g)	10.8
Lipids (g)	0.3
Carbohydrates available (g)	82.8
Starch (g)	72.2
Soluble (g)	2.7
Dietry fiber (mg)	2.6
Iron (mg)	1.3
Calcium (mg)	17.0
Phosphorus (mg)	165.0
Sodium (mg)	5.0
Potassium (mg)	160.0
Vit. B1 (mg)	0.14
Vit. B2 (mg)	0.11
Vit. pp (mg)	2.0
<b>Energy (kcal)</b>	<b>356</b>

Source: Ferrari and Piazza (2006).

Durum bread has a yellowish color, a characteristic taste and smell, a fine and uniform crumb structure, and more prolonged shelf life (Liu et al., 1996). Durum bread has also been reported to be less toxic for those who suffer from intolerance to wheat gluten (celiac disease) (Troncone and Auricchio, 1991).

## **2.5. Quality requirements of durum wheat and semolina for pasta production**

### **2.5.1. Physico-chemical characteristics**

There have been numerous studies attempting to correlate physical characteristics and also chemical composition of durum wheat and semolina with quality of pasta. Dexter et al (1987) reported on the relationship of durum wheat test weight to spaghetti quality. It was found that the inferior color and higher ash of the semolina derived from low test weight durum wheat led to inferior spaghetti color. The lone beneficial effect of low test weight was an improvement in cooked spaghetti firmness and resilience, because of a strong negative relationship between test weight and wheat protein. The other physical properties which were discussed in sections 2.2 and 2.3 regarding milling of durum wheat, can indirectly influence the quality of pasta. For example, the pasta industry considers vitreousness to be important for semolina yield, granulation, and purity and a ready indicator of sufficient protein content in durum wheat samples (Blanco et al., 1988). On the other hand, non vitreous grains are considered as a negative factor for criteria such as cooking quality and color of pasta. However, that does not mean that

semolina derived from vitreous grains will always produce pasta of good cooking quality (Cubadda, 1988).

Protein quantity and quality and their effects on pasta quality have been the subject of many research works. There is universal agreement that protein content is the primary factor influencing pasta quality and that gluten strength is an important secondary factor (Matsuo et al., 1982; Aufran et al., 1986; D'Egidio et al., 1990; Novaro et al., 1993). The SDS-sedimentation test (Dexter et al., 1980; Quick and Donnelly, 1980) is an effective rapid indicator of durum wheat gluten strength. Recently, a minimum SDS-sedimentation volume has become a popular durum wheat quality specification (Feillet and Dexter, 1998).

Wheat proteins are a highly heterogeneous material, including albumins (soluble in water), globulins (soluble in neutral salt solutions), gliadins (soluble in 70% ethanol and in acids) and glutenins (soluble in acids, bases, hydrogen, and hydrophobic bond-breaking solvents) (Feillet, 1988). Gluten is a complex mixture of gliadins and glutenins. Gliadins are heterogeneous monomeric proteins which are separated into groups on the basis of their mobility on electrophoresis at low pH, and responsible for gluten plasticity (extensibility) (Shewry et al., 1999). Different groups of gliadins, in order of increasing mobility, are the  $\omega$ -gliadins,  $\gamma$ ,  $\beta$ , and  $\alpha$ -gliadins (Shewry et al., 1999). The glutenin fraction consists of high molecular weight polymers stabilized by inter-chain disulphide bonds (Carrilo et al., 1990). On the basis of their mobility in SDS-PAGE, the single monomers are usually classified into

two groups, the high molecular weight subunits of glutenin (HMW-GS) and the low molecular weight subunits of glutenin (LMW-GS) (Galterio et al., 1991; Gupta et al., 1995). The total amount of glutenin subunits and the presence of certain subunits have shown to be associated with superior rheological characteristics of gluten, such as elasticity and dough strength (Pogna et al., 1988, 1990; Carrilo et al., 1990). In common wheat, HMW-GS are encoded by genes located at three complex loci, Glu-A1, Glu-B1 and Glu-D1 (Payne and Lawrence, 1983). Since durum is a tetraploid wheat, the 'D' genome is absent in this wheat, therefore, the subunits 2, 3, 4, 5, 10 and 12 which are coded for by genes on the 'D' genome will be absent in durum wheat (Khan and Bushuk, 1977; Payne and Lawrence, 1983; Du Cros, 1987). Many researchers (Matsuo and Irvine, 1970; Walsh and Gilles, 1971; Matsuo et al., 1972; Dexter and Matsuo, 1978b, 1980) have established that content and composition of proteins, gluten strength in particular, are important for the cooking quality of pasta. Damidaux et al (1978) showed that varieties of durum wheat that contain gamma-45 gliadin have superior cooking quality than those containing gamma-42 gliadin. Both proteins have the same molecular weight (45,000) and similar amino acid composition (Feillet, 1980). However, Payne et al (1984) and Pogna et al (1988) showed that the presence or absence of low molecular weight LMW-1 and LMW-2 glutenins are linked closely genetically to  $\gamma$ -42 and  $\gamma$ -45 gliadins respectively and these two gliadin polypeptides can be used only as genetic markers for other proteins responsible for gluten quality. Among high molecular glutenin subunits, HMW-GS 6+8 or 7+8 give better pasta cooking quality than HMW-GS 20 (Kovacs et al., 1995). Autran and Feillet (1987) found a weak but

significant relationship between HMW glutenins and pasta quality; HMW-GS 6+8 was positively associated with quality, whereas 13+16 was negatively associated. Sgrulletta and De Stefanis (1989) showed that acetic acid insoluble protein of semolina is more efficient than the total protein content for predicting pasta cooking quality.

The yellow color of pasta products, rather than cooking behavior and taste, is reported to be one of the most important considerations in assessing durum wheat quality (Borrelli et al., 1999). A high level of carotenoid pigments in durum wheat and semolina does not, however, guarantee a high color of pasta itself because pasta yellowness and pigment loss during processing are affected mainly by lipoxygenase (LOX), and also by peroxidase (POD), and polyphenol oxidase (PPO) enzyme activities (Irvine and Winkler, 1950; Kobrehel et al., 1974; Taha and Sagi, 1987; Borrelli et al., 1999). It is generally well accepted that pigment loss on making pasta and subsequent yellowness of pasta are significantly correlated with LOX activity (Mc Donald, 1979). Pasta products made from cultivars with a high POD activity is known to develop an undesirable brownish color during processing (Iori et al., 1995; Fraignier et al., 2000). In wheat based products PPO activity has been implicated in darkening reactions that limit the acceptability of certain pasta products (Singh and Sheoran, 1972). Therefore, to optimize the color of pasta product, durum wheats with high carotenoid pigment content, low PPO activity and low lipoxygenase activity should be selected (Feillet and Dexter, 1998).

A few research data are available regarding possible negative effects of protease activity on pasta quality. Petruzzelli et al (1981) found a significant and negative correlation between durum wheat protease activity and pasta cooking quality. However, proteolytic enzymes probably have no effect in determining the quality of pasta from sound wheat, but highly sprouted wheats, having high protease activity may have a negative influence on pasta quality (Feillet, 1988).

Starch constitutes about 60-70% of semolina and exists in the form of large and small granules. The diameter of these granules lies between 20  $\mu\text{m}$  and 50 $\mu\text{m}$  (Antognelli, 1980). In comparison to proteins, the amount of publications available on the role of starch in determining pasta quality is very limited (Feillet et al., 1996). Pasta cooking quality has been shown to be highly influenced by starch gelatinization and protein network formation. During pasta cooking, a weak or discontinuous protein matrix results in a protein network that is too loose and permits a greater amount of exudates to escape during starch granule gelatinization (Resmini and Pagani, 1983). The exudates form a surface starch and the pasta becomes sticky (Feillet, 1988). The firmness of cooked spaghetti must, in part, be influenced by gelatinized starch properties (Dexter and Matsuo, 1979b). Shuey and Gilles (1964) studied the gelatinization characteristics of durum semolina and durum products and found that amylographic peak values for semolina and macaroni are correlated. Lintas and D'Appolonia (1973) found that semolina starch had a higher peak viscosity than the starch isolated from spaghetti due to starch damage during processing. Further studies showed that some starch was

damaged during the extrusion phase of processing, but the greatest change occurred during the drying process (Lintas, 1988). Yue et al (1999) reported significant changes in starch properties during pasta drying. They also found that some of the quality improvements in high temperature and very high temperature dried pasta that occur must be attributed to these changes.

A part of research work on starch has been devoted to starch-protein and starch-lipid interactions. The knowledge of these phenomena can help scientists to improve pasta cooking quality. Dahle and Muenchow (1968) reported that the removal of lipids or proteins increased the amount of amylose in the cooking water. Removal of lipids led to greater stickiness of pasta. D'Egidio et al (1984) isolated a protein fraction able to form complexes with starch and found that for some wheats improvement in pasta quality can be achieved by increase in this protein fraction. However, a polypeptide on the surface of starch granules which interfere with the adhesion of the granules to the wheat protein matrix has been reported to be absent in durum wheat (D'Appolonia and Rayas-Duarte, 1994.). Feillet (1984) found that using a water with acidic pH (pH 6) for pasta cooking can prevent the leaching of starch into the cooking water because in this condition protein molecules are positively charged and starch molecules are negatively charged and starch-protein interaction is enhanced. Cooked spaghetti stickiness might be related to the level of insoluble amylose-lipid complexes on the surface of starch granules (Matsuo, 1994). Grant et al (1993) indicated that the addition of monoglyceride to semolina inhibit the loss of soluble carbohydrates, which may have a significant effect on stickiness of pasta. Spaghetti made from this



semolina had lower content of amylose because of amylose-monoglyceride complex formed during mixing and extrusion.

### **2.5.2. Rheological Characteristics**

Through chemical and rheological tests, the suitability of soft wheat for breadmaking and for other types of processing can be evaluated. In the past, various attempts were made to apply these methods, sometimes with modifications, to the evaluation of the pasta-making potential of semolina.

Irvine et al (1961) for the first time used farinograph, with some modifications, to determine macaroni dough characteristics at processing water absorptions of 26.5 to 36.0%. They suggested that changes in various semolina qualities are reflected by typical changes in farinograms and this technique can be used in continuous macaroni processing plants to control paste properties. Their technique is still being used to study the farinograph properties of semolina, and Brabender Company has developed a software based on their modifications which can be used in the new model of farinograph instrument. Matsuo and Irvine (1970) and Dexter and Matsuo (1980) also found relation between pasta cooking quality and farinograph mixing characteristics. Gluten strength which affects the firmness of cooked pasta (Walsh, 1971) can be measured by mixing characteristics of mixograph (Bendelow, 1967) which satisfactorily indicate gluten type (i.e. strong, medium or weak). However, Dexter et al (1980) claim that mixograph gives a poor prediction of cooking quality. Viscoelastograph also can be used to predict semolina gluten strength (Kovacs et al., 1994). In his review of some of the

tests used to predict durum wheat and pasta quality, Dick (1985) discussed mixograph and farinograph tests and their relevance to pasta quality.

Walle and Trentesaux (1980) studied the evaluation of durum wheat and semolina for macaroni making by using Chopin Alveograph. D'Egidio et al (1990) analyzed 50 samples of 10 Italian durum varieties by various technological and chemical tests and showed a strong relationship between alveograph properties and pasta cooking quality.

Cubadda (1988) explained that although farinograph and alveograph are able to provide information on the characteristics of semolina before extrusion, they are nevertheless inadequate for predicting the cooking quality of durum wheat semolina.

As mentioned in section 2.3, high levels of  $\alpha$ -amylase in semolina due to durum wheat sprout damage, influence the quality of the pasta product. The falling number test (Hagberg, 1961) is highly correlated with wheat  $\alpha$ -amylase activity and is a good indicator of sprout damage. High amylolytic activity in wheat increases the amount of residue in the cooking water and the level of reducing sugars in both semolina and spaghetti and tends to give a slightly softer cooked spaghetti (Matsuo, 1988).

## 2.6. Evaluation of pasta cooking quality

The ultimate test of acceptability of pasta product is its cooking quality (Matsuo, 1988). Cooking quality of pasta is of importance to consumers and thereby to wheat producers, breeders and processors (Feillet and Dexter, 1998). Proper evaluation of pasta cooking quality requires consideration of a number of factors including its elasticity, firmness, surface stickiness, cooking time, cooking tolerance, water absorption (cooked weight), and loss of solids to cooking water (Edwards et al., 1993; Feillet, 1984). The preferred pasta shape for pasta evaluation is the solid cylindrical form (spaghetti) because its geometrical form gives the best indication of the intrinsic quality of durum wheat semolina (D'Egidio and Nardi, 1998). For comparison of different samples with each other, identical cooking conditions including cooking time, hardness and pH of cooking water, as well as the time elapsed between pasta draining and testing should be taken into consideration. Cooking time influences all textural parameters; generally, minimum cooking time (optimal cooking time) is used which corresponds to disappearance of the center core of spaghetti. Sometimes normal cooking time (minimum cooking time plus 1-3 min), or a fixed standard cooking time (12-13 min) and over-cooking time (normal cooking time plus 10-12 min) are utilized to assess the cooking quality of spaghetti (D'Egidio and Nardi, 1998). Water hardness exerts high influence especially on surface stickiness (Dexter et al., 1983; Malcolmson and Matsuo, 1993), whereas time elapsed after draining influences firmness and stickiness (Voisey et al., 1978; Dexter et al., 1983).

### **2.6.1. Sensory evaluation**

Although some objective methods have been developed to evaluate the cooking quality of pasta, sensory evaluation still remains the most reliable test (Cubadda, 1988), provided certain critical points are considered in the sensory evaluation (Menger, 1979). Sensory judgment is expressed by the components: stickiness, firmness, and bulkiness (Cubadda, 1988). A standard method has been elaborated by the international standards organization (ISO) for evaluation of spaghetti cooking quality by sensory analysis (D'Egidio and Nardi, 1998). However, some difficulties occur in sensory evaluation related to the different background and experience of the testers (Troccoli et al., 2000), and also they are time-consuming and impractical when sample size is limited or large number of samples are to be evaluated (Edwards et al., 1993). In response to these constraints, a number of objective methods have been developed to evaluate the cooking quality of pasta.

### **2.6.2. Objective methods**

Cooking loss which is considered as an indicator of overall spaghetti cooking performance can be determined by weighing the residue of cooking water after evaporation or after freeze drying (Dexter and Matsuo, 1979a). Matsuo et al (1992) developed a colorimetric method measuring the absorption of the iodine-amylose complex in cooking water.

The material that can be rinsed off the surface of cooked spaghetti, called total organic matter (TOM) by some Italian researchers (D'Egidio et al., 1982, 1990, 1993), was found to be highly correlated with sensory estimation.

Stickiness can also be measured with the Grain Research Laboratory compression tester (Dexter et al., 1983; Grant et al., 1993). Values obtained with this instrument were highly correlated with TOM values (Dexter et al., 1985).

The use of the Instron Universal Testing Machine has been well established for the measurement of pasta firmness (Walsh, 1971; Oh et al., 1983). This instrument has also been recommended by American Association of Cereal Chemists (AACC).

Microscopy techniques have been used by different researchers (Dexter et al., 1978; Dexter et al., 1979; Resmini and Pagani, 1983; Pagani et al., 1986) in the micro-structural studies of cooked pasta. This tool gives a qualitative estimation of textural attributes related to size, shape and arrangement of particles (geometric characteristics).

Generally, chemical and instrumental tests, measure isolated analytical parameters; sensory procedures, instead, take many textural characteristics into account jointly. An efficient objective method should have the following characteristics (D'Egidio and Nardi, 1998):

- High correlation with sensory evaluation.
- Ease of operation and reproducibility among different laboratories.
- The ability to discriminate samples with little quality differences (high sensitivity).

### 3.1. Materials

#### 3.1.1. Durum wheat varieties

Fourteen Indian durum and two aestivum wheat varieties used in this study were obtained from the following sources:

Wheat variety	Source
DWR 2006	University of Agricultural Sciences, Dharwad
MACS 1967 MACS 2694 MACS 2846 MACS 3125 HD 2189* HD 2781*	Agharkar Research Institute, Pune
PDW 233 PDW 274 PDW 215 WH 896	Punjab Agricultural University, Ludhiana
NIDW 15 NIDW 295 N 59 Raj 1555	Agricultural Research Station, Niphad
HI 8498	Indian Agricultural Research Institute, Indore

\* Aestivum varieties

Two Italian durum wheats, namely, Lira 45 and Lira 42, which are biotypes of the variety Lira, were obtained for comparison studies in some experiments. All wheat varieties were stored in cold storage (4-6 °C) until further use.

### 3.1.2. Chemicals and Enzymes

Various chemicals used in the present investigation were procured from the following sources:

Sodium dodecyl sulphate, Coomassie brilliant blue R-250, bromophenol blue, Tris, acrylamide, N,N-methylene bis acrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED), trifluoro acetic acid (TFA),  $\beta$ -mercapto ethanol, *o*-dianisidine, catechol, linoleic acid, azocasein and standard proteins (carbonic anhydrase, ovalbumin, bovine plasma albumin, rabbit muscle phosphorylase B, rabbit muscle myosin, *E. coli*  $\beta$ -galactosidase) were obtained from Sigma Chemical Company, St. Louis, USA. Glycine and H<sub>2</sub>O<sub>2</sub> were procured from Qualigens Fine Chemicals, Mumbai, India. HPLC grade acetonitrile was obtained from Merck Ltd., Mumbai, India. All other chemicals, reagents and solvents used in the present study were of analytical grade and obtained from reputed companies.

Microbial lipase which is a purified lipase from *Aspergillus oryzae* was a gift from Novozymes, South Asia Pvt. Ltd., Bangalore, India. This enzyme had an activity of 300 KLU/g (Kilo Lipase Units/g).

Microbial transglutaminase derived from *Streptovercillium* sp. was a gift from Ajinomoto Co., Inc., Japan. This enzyme had an activity of 100 U/g.

## 3.2. Methods

### 3.2.1. Durum wheat

#### 3.2.1.1. Physico-chemical properties of durum wheat

(a) **Hectoliter weight** of different durum wheat varieties was determined according to AACC standard method (2000).

(b) **1000-kernel weight** was determined using a seed counter (Numigral, Falling Number AB, Stockholm, Sweden). A representative sample of each variety was taken in triplicate and the average value is reported.

(c) **Hardness** of durum wheat kernels was measured using 'Instron' universal testing machine equipped with a special rod shape plunger with 50 mm diameter (Model 4301, High Wycombe, UK). Fifty sound kernels of each variety were taken randomly and the force required to crush individual kernels was noted and the average value calculated and expressed in terms of Newton (N). The crosshead speed of the plunger was maintained at 100 mm/min with a load cell of 500 kgf at 50% compression.

(d) **Length and Breadth** of the kernels were determined by placing the kernels either horizontally or vertically, on a graph paper in such a way that the edge of one kernel touched the edge of the next kernel. For each measurement, 10 kernels were taken and average value of three experiments is reported in mm.

(e) **Vitreousness** of durum wheat kernels was determined by visual examination of 100 kernels and the experiment was carried out three times. Average value was expressed in terms of percent vitreousness.

(f) **Moisture, ash, protein (Nx5.7), and falling number** were determined according to AACC standard methods (2000). Falling number was carried out



using Falling Number 1400 (Type SGM 65/60-6 A120, Sweden).

**(g) Yellow pigment content** of durum wheat varieties was determined according to AACC standard method (2000) with slight modification. Wheat samples were ground to pass through 60-mesh (250 $\mu$ ) sieve. Eight grams (on 14% mb) of the ground wheat was transferred to 250 ml stoppered flask to which 40 ml of water saturated n-butyl alcohol was added. Contents were shaken for 1 min and let stand for 16 h for extraction at room temperature. After completion of standing time, contents were shaken again, filtered through Whatman No. 1 filter paper into a test tube. Absorbance of the above extract was read at 435.8 nm using a Shimadzu UV-Vis spectrophotometer (UV-160A). The pigment content of the extract (ppm) was calculated by multiplying the absorbance reading using the conversion factor 30.

**(h) Determination of Peroxidase (POD), Polyphenol oxidase (PPO), Lipoxygenase (LOX) and Protease activities in durum wheat varieties**

**Enzyme Extraction:** Whole wheat flour (1.0 g) was suspended in 10.0 ml of 50 mM sodium phosphate buffer (pH 7.5) and stirred with constant agitation on a magnetic stirrer for 2 h at 4 °C. The suspension was centrifuged at 8000g in a refrigerated centrifuge for 15 min. The clear supernatant obtained was used for the analysis of POD, PPO, LOX, and protease.

**Determination of pH optimum of POD, PPO and LOX enzymes:** To find the pH optimum of POD, PPO and LOX enzymes, the enzyme extracts were assayed using different buffers. Sodium acetate buffer and sodium phosphate buffer

were used for pH 3.5-6.5 and pH 6.5-8.0, respectively. The pH optimum for POD, PPO and LOX was found to be 5.0, 7.5 and 5.5, respectively. Further enzyme assays were carried out at each of their optimum pH conditions. All enzyme assays were carried out at room temperature.

**Measurement of enzyme activity:** POD activity was measured using a reaction mixture containing 50 mM sodium acetate buffer (pH 5.0; 750  $\mu$ L), 1% H<sub>2</sub>O<sub>2</sub> (100  $\mu$ L), 0.25% o-dianisidine (100  $\mu$ L) and enzyme extract (50  $\mu$ L). The increase in absorbance was monitored at 460 nm for 120 s using a Shimadzu UV-vis Spectrophotometer (UV – 160A). One unit of enzyme activity is defined as the change in absorbance of 1.0 per min (Aparicio – Cuesta et al., 1992). The reaction mixture to measure PPO contained 50 mM sodium phosphate buffer (pH 7.5; 800  $\mu$ L), 0.5 M catechol (100  $\mu$ L) and enzyme extract (100  $\mu$ L). The increase in absorbance was monitored at 420 nm for 120s using a Shimadzu UV-vis spectrophotometer (UV – 160A). One unit of enzyme activity is defined as the change in absorbance of 0.1 per min (Coseteng and Lee, 1987).

To measure LOX activity, linoleic acid substrate was prepared according to Shiiba et al (1991). The reaction mixture consisted of 0.2 M sodium acetate buffer (pH 5.5; 870  $\mu$ L), linoleic acid substrate ( $7.5 \times 10^{-3}$  M, 30  $\mu$ L) and enzyme extract (100  $\mu$ L). Enzyme activity was expressed in terms of hydroperoxide formed ( $\mu$ mol) per min using an extinction value of  $2.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ .

Protease assay was conducted as described by Sarath et al (1989) with some modifications. Azocasein substrate (25 mg) was dissolved in 1 mL of 50 mM sodium phosphate buffer (pH 7.5). Sodium phosphate buffer (50mM, pH 7.5; 450  $\mu$ L), was added to 50  $\mu$ L of substrate solution and pre-incubated for 10 min at 37 °C. Enzyme extract (200  $\mu$ L) was added and the mixture was incubated for 30 min at 37 °C. The reaction was terminated by adding 0.5 mL of 10% TCA and the precipitate removed by centrifugation at 8000g in a refrigerated centrifuge for 10 min. NaOH (40  $\mu$ L, 10 M) was added to the supernatant and the absorbance was read at 440 nm using a Shimadzu UV-vis spectrophotometer (UV – 160A). One unit of protease activity is defined as the amount of enzyme required to produce absorbance change of 1.0 under the assay conditions.

#### **3.2.1.2. Durum Wheat Milling**

Durum wheat samples were milled into semolina using a Buhler laboratory mill (MLU 202) (Fig. 7). This instrument has a simple flow comprising of three break and three reduction passages. The three break passages are built on one roll pair and the three reduction passages are built on a second roll pair. Buhler laboratory mill works under a constant break roll differential of 2:1, fast roll speed of 500 rpm and slow roll speed of 250 rpm. The milling process was according to the method explained by Rahim et al (1974) with some modifications. Semolina milling conditions were standardized after several trials in order to obtain maximum semolina yield with optimum particle size.

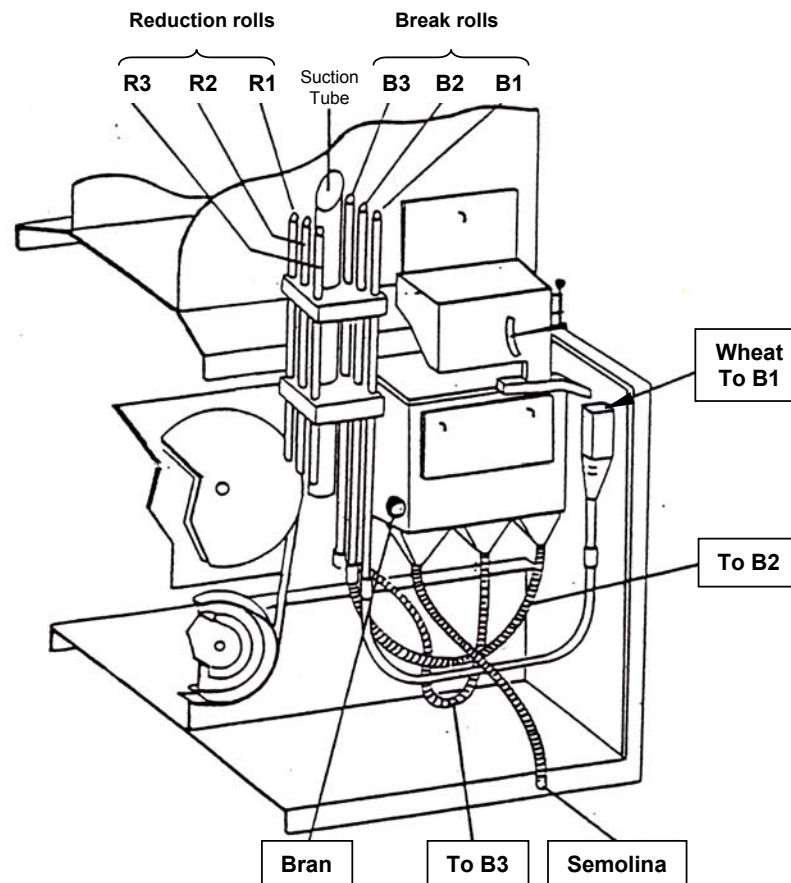
Five-kilogram batch of wheat was conditioned for 18 h at 17% moisture prior to milling. Only the break system of the mill was used by disconnecting the pneumatic conveyor pipe leading to the reduction roll system (Fig. 8). Break roll gaps of 0.4 mm and 0.2 mm for first break ( $B_1$ ) and third break ( $B_3$ ), respectively, were found optimum for getting the desired semolina. The combined overtailings from the sieves at break rolls  $B_1$ ,  $B_2$  and  $B_3$  were collected as semolina fraction. The flour and the bran obtained from different break rolls were also collected separately.

#### 3.2.1.3. Purification of Semolina

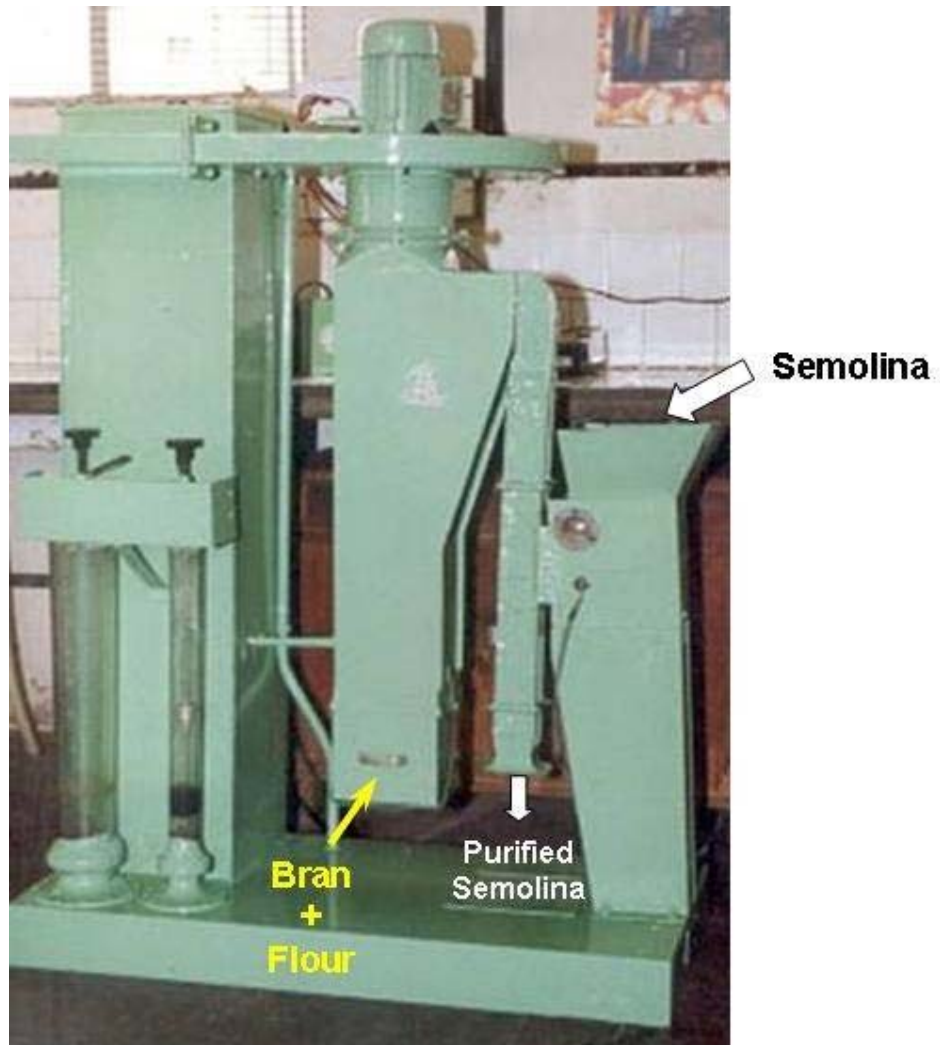
Bran contamination in semolina was removed using an air classifier (PETKUS, Germany) (Fig. 9) having air speed adjustability from 0-200 m<sup>3</sup>/h. To increase the efficiency of the purification process and to reduce the semolina loss, semolina was passed through 44 GG (425  $\mu$ ) sieve. Overtailing of 44 GG was purified using an air speed of 25 m<sup>3</sup>/h with several passings. Throughs of 44 GG was purified with an air speed of 15 m<sup>3</sup>/h with several passings. The above purification process which was standardized after several trials removed most of the bran particles present in semolina. After purification, the two fractions (+44GG and -44GG) were mixed thoroughly to get a homogenous product. Purified semolina was stored in cold storage (4-6 °C) for further studies.



Fig. 7. Buhler laboratory mill



**Fig. 8.** Schematic diagram of the back lower half of the break side of the Buhler laboratory mill modified for semolina milling (Source: Black and Bushuk, 1967 with modifications).



**Fig. 9.** PETKUS air classifier for purification of semolina

### 3.2.2. Durum Semolina

#### 3.2.2.1. Physico-chemical characteristics of semolina

(a) **Particle size distribution of semolina:** Granularity or particle size distribution of semolina was determined according to AACC standard method (2000). Five sieves, namely, 30 (595 $\mu$ ), 40 (425 $\mu$ ), 60 (250 $\mu$ ), 80 (180 $\mu$ ) and 100 (150 $\mu$ ) mesh, respectively were used in this study. Five sieves were stacked with a tared pan beneath the bottom sieve and a lid on top. 100 g of semolina was weighed and placed on the top sieve (30 mesh) of the stack and the lid was placed on the sieve. Sample was sieved for 5 min using a Buhler laboratory sifter (model KBF 7 SN, Switzerland) running at 180 rpm (Fig. 10). After running the sifter for 5 min, the overtailings on each sieve were weighed, and the result was expressed as percentage.

(b) **Semolina Color Characteristics:** Semolina color was measured as per the method of Manthey and Hareland (2001) using a colorimeter (Minolta CM, 3500d, Japan) under visible wavelength. Entire viewing area of colorimeter was covered with a layer of semolina and the sample was held firmly over viewing area with black (0%) reflectance cover. The color values of the samples were measured by C illuminating 2° view angle (CIE color scale) which gives L\* value (brightness, 100=white; 0= black) and b\* value (+, yellow; -, blue).

(c) **Moisture, ash, protein (Nx5.7), wet gluten and yellow pigment content** were determined according to AACC standard methods (2000) as described earlier for durum wheat in section 3.2.1.1.





**Fig. 10.** Buhler laboratory sifter used for determination of semolina particle size

(d) **Acetic Acid Insoluble protein of semolina:** Acetic acid insoluble protein of semolina samples was determined according to the method explained by Sgrulletta and De Stefanis (1989). Two grams of the semolina samples were extracted in 10 ml of 0.1 M acetic acid for 60 min at room temperature. The suspension was stirred four times every 15 min and centrifuged at 8000g for 20 min. A measured portion of the supernatant was used for determining the protein content (Nx5.7) by micro kjeldahl method (AACC, 2000). The insoluble protein residue, expressed in terms of percentage dry matter (% dm), was calculated as the difference between the total protein content of semolina and acetic acid – soluble fraction.

#### **3.2.2.2. Farinograph characteristics of semolina**

Farinograph characteristics of semolina were determined using Brabender Farinograph-E (Brabender OHG, Duisburg, Germany) according to the method standardized by Irvine et al (1961). Compared to the earlier models, Farinograph-E has the benefit of having a fully electronic measurement system and a computer controlled operation. The testing parameters are fed through the computer keyboard. The measuring data are transmitted via a USB communication port directly to the PC for evaluation. After the test, the instrument stops automatically. Zero point setting is also done automatically after starting of a new test.

Since the amount of water, which is used for semolina testing, is in the range of 26-36%, a modified software version 2.3.2, specific for the measurement of farinograph mixing properties of semolina, was used in this

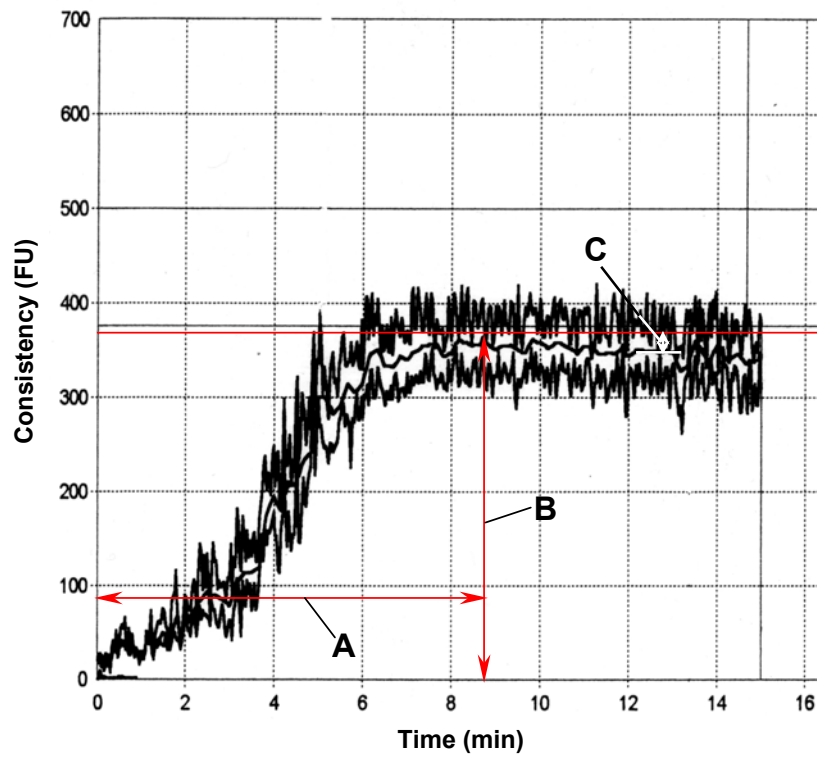
study. To comply with this software system, the type of mixer in test parameters window was set on 50 SEM to work using the 50 g bowl in the measuring range of 300 g bowl. This arrangement is equivalent to the use of the 300 g farinograph bowl, which was used at lever position of 1:3 in the earlier farinograph models.

Based on the requirements of the farinograph test,  $50.0 \pm 0.01$  g (on 14% mb) of semolina was placed in the farinograph mixer bowl. The farinograph working temperature was maintained at 30 °C. The farinograph containing the sample was started and after 1 min dry mixing, the required amount of water (35%) was added. The farinograms were analyzed for the following parameters. A sample farinogram is shown in Fig 11.

- I. Dough Development Time (DDT): The time from zero to the point of maximum consistency of the dough immediately before the first indication of weakening.
- II. Maximum Consistency (MC): Height in Farinograph units (F.U.) at the center of the curve at peak consistency.
- III. Mixing Tolerance Index (MTI): The difference in Farinograph units between the top of the curve at the peak (Maximum Consistency) and the top of the curve measured 4 min after the peak was reached.

### **3.2.2.3. Pasting properties of semolina**

Pasting properties of semolina samples were determined using Brabender Micro Visco-Amylograph (MVA) (Brabender OHG, Duisburg, Germany). The amylograms were evaluated by the software program and results displayed in



**Fig. 11.** A sample Farinogram illustrating its interpretations:

**A** – Dough Development Time

**B** – Maximum Consistency

**C** – Mixing Tolerance Index (4 min after peak)

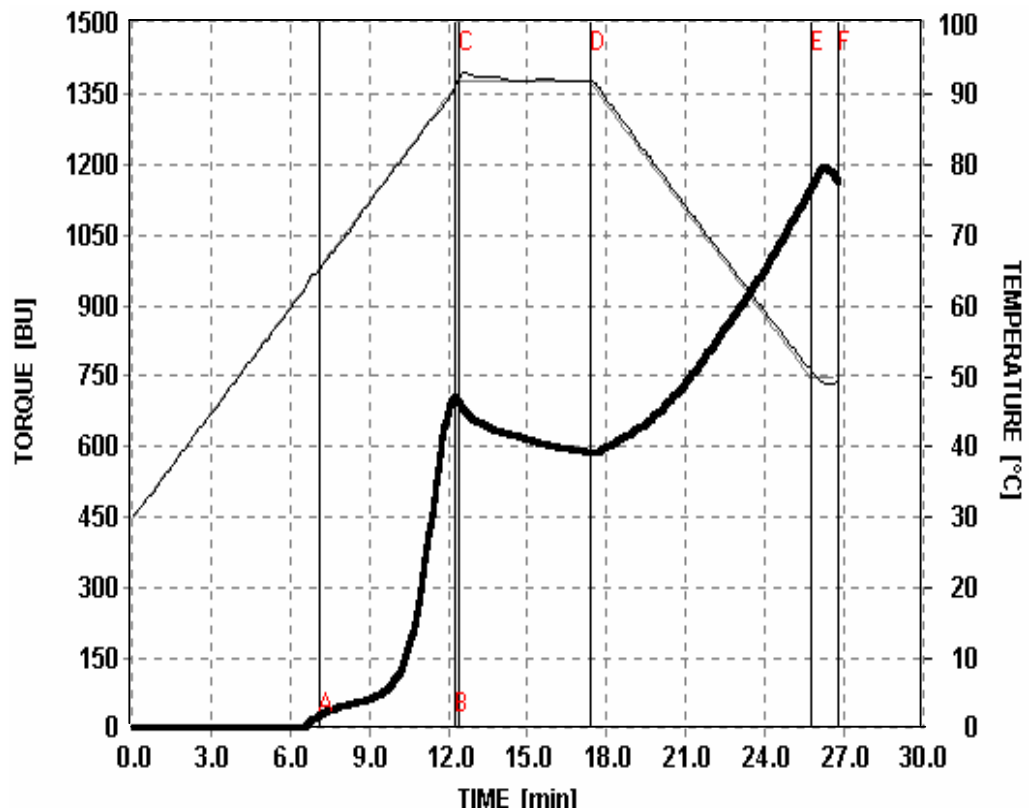
a tabular form. The MVA is equipped with an integrated, self-optimizing temperature control unit that together with the Windows software, permits programming and running of any temperature profiles. The MVA allows heating/cooling rates ranging from 1.5°C/min to 10°C/min. The PC, which is connected via USB, takes over the measured values on line, evaluates them, and displays them on-line as a numerical and graphical form. A specimen diagram illustrating the interpretation of the MVA is shown in Fig. 12.

The parameters and temperature profile used for the study were:

Speed	250 rpm	Measuring range	300 cmg
Start temperature	30 °C	Heat/cool rate	5 °C/min
Maximum temperature	93 °C	Upper Holding time	5 min
End temperature	50 °C	Final Holding time	1 min

The MVA studies were carried out as follows:

1. 100 ml of distilled water (corrected to 14.0% moisture basis) was filled into the Erlenmeyer flask supplied with the instrument.
2. 15.0 g (14.0% mb) of semolina powdered using a Laboratory Mill (Type 3100, Perten Instruments, Huddinge, Sweden) was added through the hopper into the Erlenmeyer flask.
3. The suspension was shaken several times until free from lumps
4. The suspension was filled into the measuring bowl
5. The measuring bowl was mounted onto the instrument



### Evaluation

Point	Name	Time [HH:MM:SS]	Torque [BU]	Temperature [°C]
A	Beginning of gelatinization	00:07:10	30	65.7
B	Maximum viscosity	00:12:15	698	91.3
C	Start of holding period	00:12:24	686	92.4
D	Start of cooling period	00:17:24	581	92.0
E	End of cooling period	00:25:48	1164	50.8
F	End of final holding period	00:26:48	1167	49.2
B-D	Breakdown		117	
E-D	Setback		572	

**Fig. 12.** A sample Micro Visco-Amylogram and evaluation data illustrating its interpretation

### 3.2.3. Spaghetti

#### 3.2.3.1. Production of spaghetti

Optimum level of water for mixing with semolina from individual wheat varieties was determined subjectively after several trials. Optimum level of water depended on the water required to mix it with semolina in order to produce a uniform granular mixture, which in turn produced spaghetti strands without any specks. Care was also taken not to add excess water which would otherwise have adversely affected the extrusion and drying of the extruded dough. The following steps that are shown in Fig. 13, were followed for the production of spaghetti:

**I. Pre-mixing:** Semolina was mixed with precalculated amount of distilled water (40° C) in a Hobart mixer (Model N-50, Ontario, Canada) (Fig. 14) at beater speed 1 (61 rpm) for 5 min to make a 500 g dough. The amount of water and semolina used for mixing was calculated based on the following equations (Mondelli, 2000):

$I$  = Total weight of dough (g)

$U_i$  = Moisture of dough (%)

$A$  = Total weight of water in the dough (g)

$S$  = Weight of semolina required to make the dough according to desired final moisture of the dough ( $U_i$ ) and to moisture of semolina ( $U_s$ )

$A_a$  = Weight of water to be added to semolina to make dough with desired moisture  $U_i$ , according to semolina's specific moisture ( $U_s$ )

$K$  = Semolina moisture coefficient

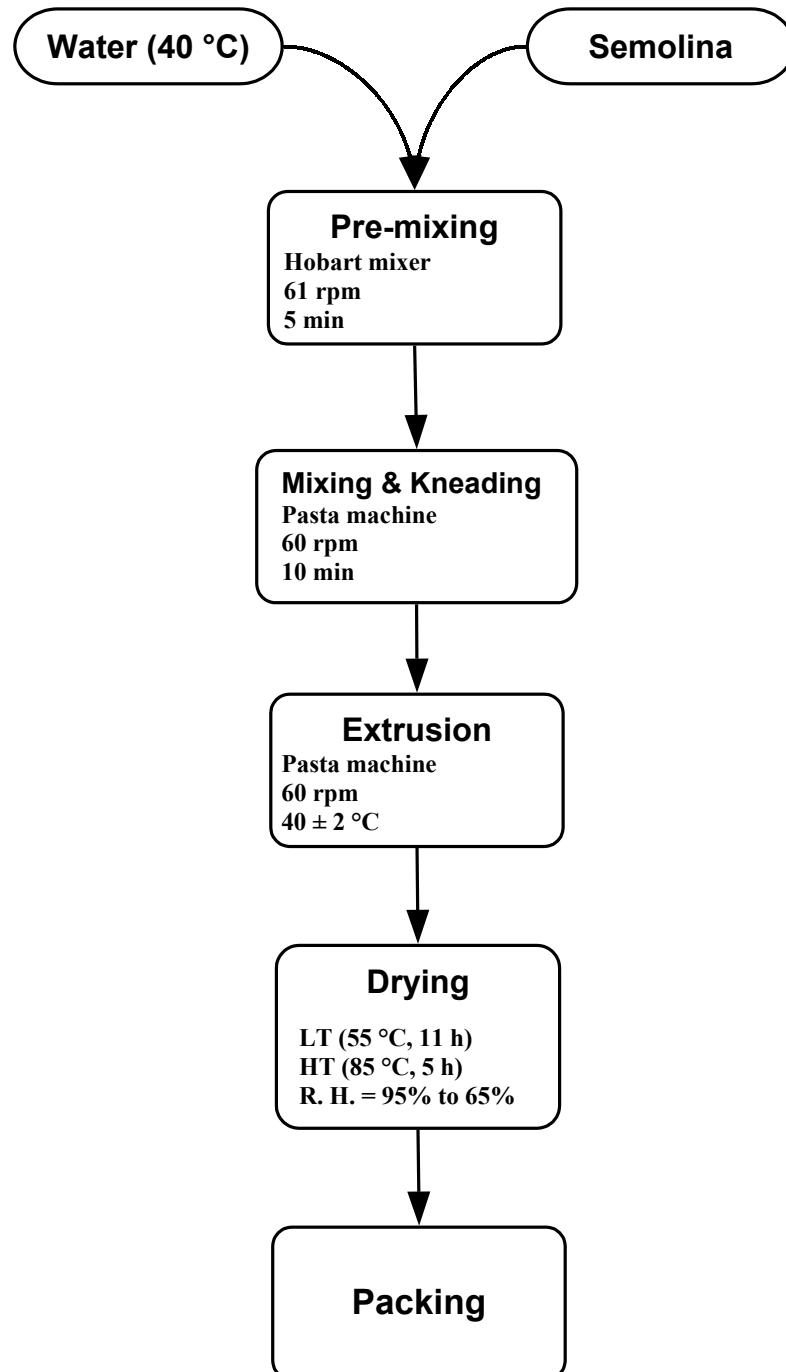


Fig. 13. Flow chart of spaghetti production



$$A = \frac{U_i I}{100} \quad K = \frac{U_s}{100} \quad S = (I - A) + \frac{(I - A)K}{1 - K} \quad Aa = A - \frac{(I - A)K}{1 - K}$$

Following the above equations, an example for the preparation of a 500 g dough with a final moisture content of 35%, and made from semolina having 13% moisture has been cited below:

$$A = \frac{35 \times 500}{100} = 175g \text{ (total weight of water in the dough)}$$

$$K = \frac{13}{100} = 0.13 \text{ (semolina moisture coefficient)}$$

$$S = (500 - 175) + \frac{(500 - 175) \times 0.13}{1 - 0.13} = 373.5g \text{ (semolina required)}$$

$$Aa = 175 - \frac{(500 - 175) \times 0.13}{1 - 0.13} = 126.5g \text{ (water required)}$$

The premixing of semolina and water in Hobart mixer resulted in a more uniform distribution of water and helped in producing spaghetti with uniform appearance and without specks.

**II. Mixing and Kneading:** The pre-mixed mixture of semolina and water was transferred to a laboratory pasta machine (La Monferrina, Model Dolly, Asti, Italy)(Fig. 15) and was mixed for 10 min at 60 rpm. Kneading of the mixture was performed by the extrusion screw or extrusion worm present in the pasta machine rotating in opposite direction of extrusion process. The kneading process imparted continuous pressure on the dough compressing just enough for it to withstand the extrusion and the shaping process.



**Fig. 14.** Hobart mixer used for premixing of semolina and water to make a uniform dough



**Fig. 15.** A view of the laboratory pasta machine

**III. Extrusion:** The dough was extruded using single screw laboratory pasta machine (Fig. 15). The temperature of extruded dough was  $40 \pm 2$  °C. A 36 strand, 1.7 mm diameter die was used to shape the dough and obtain the spaghetti strands.

**IV. Drying:** The extruded spaghetti strands were hung on wooden sticks and placed in the dryer (model Sakav, Mumbai, India). Two different drying cycles were followed for drying of spaghetti according to Zweifel et al (2000) with some modifications:

**(a) Low Temperature (LT):** In LT drying, a temperature of 55 °C was used for drying the spaghetti. Relative humidity of drying chamber was progressively reduced from 95% to 65% during 11 h of drying period. LT drying cycle is shown in Fig. 16.

**(b) High Temperature (HT):** In HT drying, a temperature of 85 °C was used for drying of spaghetti. Relative humidity of drying chamber was progressively reduced from 95% to 65% during 5 h drying period. HT drying cycle is shown in Fig. 17.

Dried spaghetti was cooled to room temperature and packed in polypropylene pouches to be used for different experiments. At least 10 days gap was maintained between drying and cooking of spaghetti to allow the dried sample to stabilize under ambient conditions (Sgrulletta and De Stefanis, 1989).

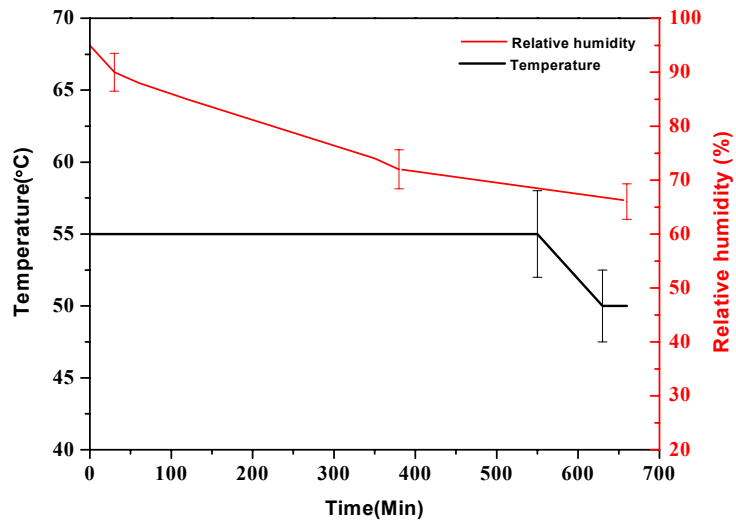


Fig. 16. Low temperature (LT) drying cycle for drying of spaghetti

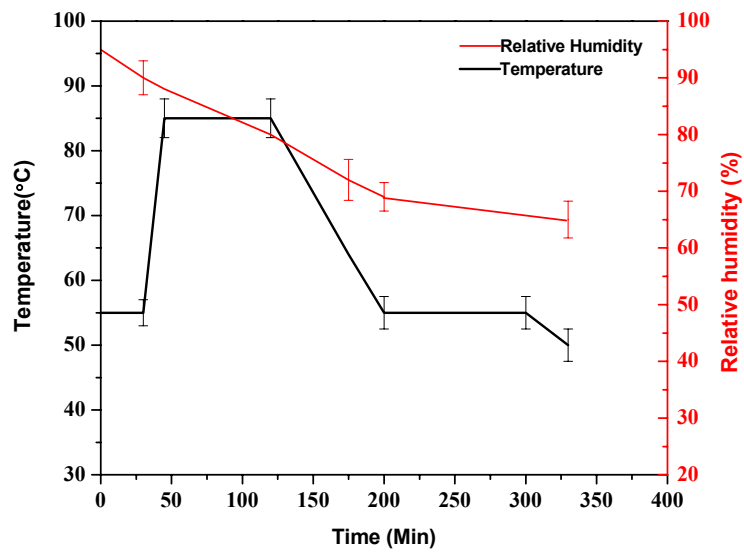


Fig. 17. High temperature (HT) drying cycle for drying of spaghetti

### 3.2.3.2. Physico-chemical characteristics of dry spaghetti

(a) **Spaghetti strength:** Strength of dried spaghetti was measured using a universal texture measuring system (LLOYDS Instruments, LR-5K, UK)(Fig. 18) with special fixtures (Fig. 19) for breaking of spaghetti strand. Spaghetti strength was expressed as force (gf) required to break one strand of dried spaghetti. The conditions used throughout the experiment included a cross head speed of 10 mm/min and a load cell of 5 kg.

(b) **Color characteristics:** Spaghetti color characteristics were measured according to the method of Manthey and Hareland (2001) using a Minolta colorimeter (Minolta CM, 3500d, Japan) under visible wavelength. Entire viewing area of colorimeter was covered with a layer of spaghetti strands and the sample was held firmly over viewing area with black (0%) reflectance cover. Color of spaghetti samples was measured by D<sub>65</sub> illuminating 10° view angle (HUNTER system) which gives 'L' value (brightness, 100=white; 0=black), 'b' value (+, yellow; -, blue), and 'a' value (+, red; -, green).

(c) **Yellow pigment content:** Yellow pigment content of powdered spaghetti samples was determined according to AACC standard method (2000) as described earlier for durum wheat in section 3.2.1.1.

### 2.2.3.3. Spaghetti cooking quality

To evaluate the cooking quality of spaghetti samples, 10 g of raw spaghetti was broken into lengths of ~5 cm and cooked in 200 ml of boiling distilled water for 10 min according to Bureau of Indian Standards (BIS) method (IS 1485, 1993). Cooking tolerance of spaghetti samples was determined by evaluating different parameters in samples cooked for 20 min.



Fig. 18. LLOYDS texture measuring system



**Fig. 19.** Aluminum probe attached to LLOYDS Texture analyzer for measurement of spaghetti strength

**(a) Cooking loss (total solids in gruel)**

Cooking loss of different spaghetti samples was carried out according to BIS method (IS 1485: 1993) with some modifications.

Ten grams of spaghetti was broken into lengths of ~ 5 cm and was cooked in 200 ml of boiling distilled water for a period of 10 min with occasional stirring. After cooking, the sample was drained and rinsed with stream of distilled water (~ 50 ml, room temperature) for about 30 s on a Buchner funnel and allowed to drain for 2 min. Total volume of the gruel and the rinsed water collected was measured. The gruel was shaken well for even distribution of the solid content. Twenty milliliters of the above gruel was pipetted out into a tared petri dish and evaporated to dryness on a water bath. The petri dish was transferred to a hot air oven maintained at  $105 \pm 2^\circ\text{C}$  and dried to constant mass.

$$\text{Total solids in gruel, percent by mass} = \frac{(M_2 - M_1)V}{2}$$

where,

$M_2$  = Mass, in g of petri dish with total solids

$M_1$  = Mass, in g of empty petri dish

V = Volume of gruel in ml

**(b) Cooked Weight:** Cooked weight was determined by weighing the drained and rinsed spaghetti and reported in grams.

**(c) Spaghetti Firmness:** Spaghetti firmness was measured according to the method described by Walsh and Gilles (1971) with slight modifications using a



universal texture measuring system (LLOYDS Instruments, LR-5K, UK). Cooked spaghetti samples were immediately transferred to a 250 ml beaker containing distilled water, at room temperature. Two cooked spaghetti strands, at a time, were removed from the water and sheared within 0.5 mm distance from the base plate at a 90° angle using a specially designed aluminum shearing blade with a contact surface of 1 mm (Fig. 20). The shear was performed at a cross head speed of 10 mm /min and a load cell of 5 kg. The force (gf) required to shear the spaghetti was measured in triplicate and the average value was reported. A higher shear value indicates a firmer product.

**(d) Spaghetti Stickiness:** Surface stickiness of the cooked spaghetti was determined according to Dexter et al (1983) with some modifications according to Grant et al (1993). A universal texture measuring system (LLOYDS Instruments, LR-5K, UK) with special plunger and sample holder (Fig. 21) was used for the measurement of spaghetti stickiness.

The fixtures attached to the texture measuring instrument for evaluation of spaghetti stickiness comprised of three different parts which were fabricated at CFTRI based on the model suggested by Dexter et al (1983). These parts were, a polished aluminum plate (100x100x6 mm) as base plate, an aluminum plate (100x100x6 mm) as sample holder with a rectangular opening (43x22 mm) for plunger-to-sample access, and a polished aluminum plunger (40x19 mm contact surface). Ten grams of raw spaghetti were broken into ~5-cm long strands and cooked for 10 min.



**Fig. 20.** Aluminum shearing blade attached to LLOYDS texture analyzer for measurement of spaghetti firmness

Cooked spaghetti was drained (not rinsed) for 1 min over a Buchner funnel and loaded onto the polished aluminum base plate. The strands of cooked spaghetti were laid side-by-side to cover an area of about 5 cm. The plate was placed in a zip lock plastic bag during the interval between loading and testing to prevent the sample from drying.

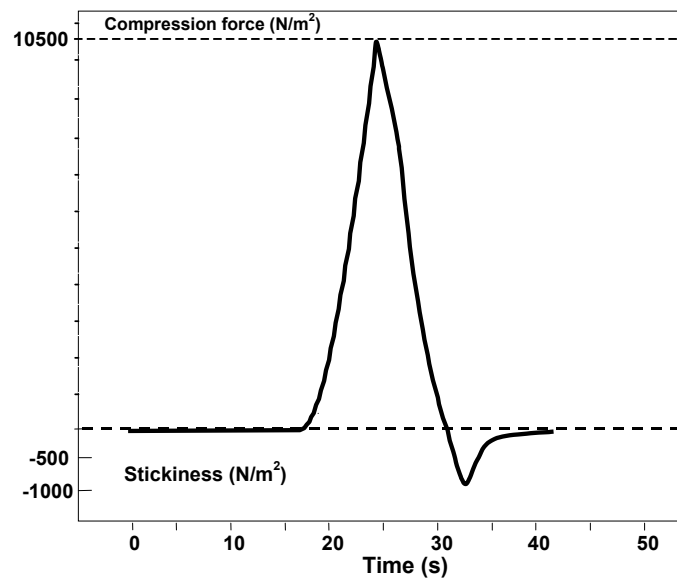
Stickiness test was started 13 min after the end of cooking. One minute before testing, sample holder plate was placed over the spaghetti strands and excess water was blotted using tissue. The plunger was fastened to the instrument cell, which was attached to a 5-kg load transducer. The plunger was moved vertically with a computer software program. The conditions used throughout the testing included a cross-head speed of 4 mm/min, and a compression force of 8 N (~800 g). The force generated during the compression of the cooked spaghetti, and on lifting of the plunger was graphically plotted on the computer screen (Fig. 22). Stickiness was defined as the maximum depression (negative value) recorded during lifting of the plunger. The negative value was calculated into  $\text{N/m}^2$  using a factor of 10500  $\text{N/m}^2$  for a compression force of 8 N (~ 800 g).

#### **3.2.3.4. Pasting properties of spaghetti**

Raw spaghetti and freeze-dried cooked spaghetti samples were ground using a laboratory mill (Type 3100, Perten Instruments, Huddinge, Sweden). Starch pasting properties of the ground samples were measured according to the procedure explained for pasting properties of semolina (section 3.2.2.3) using a micro visco-amylograph.



**Fig. 21.** LLOYDS texture analyzer with special plunger and sample holder for instrumental assessment of cooked spaghetti stickiness



**Fig. 22.** Typical graph illustrating the instrumental assessment of cooked spaghetti stickiness

### 3.2.4. Sensory evaluation of spaghetti samples

Sensory characteristics of the cooked spaghetti samples were evaluated by a panel of five experts according to the method of Cubadda (1988). The textural properties evaluated were stickiness (the state of surface disintegration of cooked spaghetti that is related to material adhering to the surface of cooked spaghetti), firmness (the resistance of cooked spaghetti when chewed or flattened between the fingers or sheared between the teeth), and bulkiness (the degree of adhesion of spaghetti strands after cooking). The sensory scores ranged from 0 to 100 as seen in Table 4. Spaghetti was evaluated by sensory procedure 9 min after draining (D'Egidio et al., 1993; Marconi et al., 1999). The overall value of the cooking quality was obtained by summing the score for each characteristic, multiplying the sum by 33.3 and dividing by 100. The final value of the cooking quality obtained from the mean of the scores given by all the panelists was correlated to a description. Spaghetti with a total score of  $\leq 40$  was of poor quality;  $> 40$  to  $\leq 50$  was not completely satisfactory;  $> 50$  to  $\leq 70$  was fair;  $> 70$  to  $80$  was good;  $> 80$  was excellent.

**Table 4.** Sensory evaluation of the quality of cooked spaghetti

<b>Score</b>	<b>Stickiness</b>	<b>Bulkiness</b>	<b>Firmness</b>
<b>0</b>	Totally	Totally	Absent
<b>20</b>	Very high	Very high	Rare
<b>40</b>	High	High	Insufficient
<b>60</b>	Rare	Rare	Sufficient
<b>80</b>	Almost absent	Almost absent	Good
<b>100</b>	Absent	Absent	Excellent

### 3.2.5. SDS-PAGE studies

Gluten proteins were extracted from flour and fractionated by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS). Vertical slab gel electrophoresis was carried out with the use of electrophoresis apparatus. A gel of 1.5 mm thickness and 14 cm length was used (2 cm stacking gel and 12 cm separating gel) according to the procedure of Laemmli (1970) as modified by Du Cros (1987).

#### 3.2.5.1. Preparation of tank buffer

Tris-glycine buffer (pH 8.3) was prepared by dissolving 3.0 g of Tris (0.025 M), 14.4 g of glycine (0.192 M) and 1.0 g of SDS (0.1%) in 1.0 L of water.

#### 3.2.5.2. Preparation of stock solutions

(a) **Monomer Stock Solution:** Acrylamide (29.2 g) and bisacrylamide (0.8 g) were dissolved in about 70 ml of distilled water and volume was made up to 100 ml. This solution was filtered and stored at 4 °C in a brown bottle.

(b) **Separating gel buffer:** Tris (13.98 g) was dissolved in 30 ml of distilled water (2.31 M) and the pH adjusted to 8.8 with HCl and the volume made up to 50 ml. This solution was stored at 4 °C for further use.

(c) **Stacking gel buffer:** Tris (3.0 g) was dissolved in 45 ml of water and the pH adjusted to 6.8 with HCl and the volume made up to 50 ml. This solution was stored at 4 °C for further use.

(d) **Sample buffer:** Two milliliters of glycerol, 0.5 ml of  $\beta$ -mercaptoethanol, 4 ml of 10% SDS and 1.25 ml of Tris-HCl buffer (0.5 M, pH 6.8) were mixed

thoroughly and volume was made up to 10 ml with distilled water. This solution was 20%, 5% and 4% with respect to glycerol,  $\beta$ -mercaptoethanol and SDS, respectively, and 0.06 M with respect to Tris. To this solution, 0.01% bromophenol blue was added as tracking dye.

### 3.2.5.3. Preparation of separating gel solution

The monomer stock solution was diluted to give 10% or 12% gel solution. For 10% gel, 9 ml of monomer stock solution, 10.64 ml of water, 6.79 ml of Tris buffer (pH 8.8), 270  $\mu$ l of 10% SDS and 270  $\mu$ l of freshly prepared (100 mg/ml) ammonium persulphate (APS) in water were mixed together by gentle swirling. To this solution, 27  $\mu$ l of TEMED was added and the mixture was poured between the assembled two glass plates. Top of the gel was covered with a thin layer of water. For the preparation of 12% gel, 10.8 ml of monomer stock solution and 8.84 ml of water were used. The 10% gel was used for examination of the HMW glutenin proteins and 12% gel was used for LMW proteins.

### 3.2.5.4. Preparation of stacking gel solution

The monomer stock solution was diluted to give 5% gel solution. 1.3 ml of monomer stock solution, 4.15 ml of water, 1.88 ml of Tris buffer (pH 6.8), 75  $\mu$ l of 10% SDS and 75  $\mu$ l of freshly prepared (100 mg/ml) ammonium persulphate in water were mixed together by gentle swirling. Then 22.5  $\mu$ l of TEMED was added and the solution was shaken gently and quickly poured between the plates, on top of the separation gel. Before the addition of

stacking gel, water layer on top of the polymerized separating gel was removed carefully with a syringe. Immediately after introducing the stacking gel solution, a well former (comb) was inserted between the plates. After the polymerization and before loading of the sample, formed wells marked with a marker and well former was removed carefully.

#### **3.2.5.5. Sample preparation and application**

Several wheat kernels from each variety were ground into powder using a mortar and pestle. Total protein of ground samples was extracted with sample buffer (25 mg flour/400  $\mu$ l buffer) in an eppendorf tube. Extraction was done for 30 min by occasional vortexing. The sample tubes were placed in a boiling water bath for 5 min and then centrifuged at 8000g. Supernatant was carefully taken for loading on the gel. Both the electrophoresis tanks were filled with tank buffer and 30  $\mu$ l of each sample was layered carefully in each well. The electrophoresis was carried out initially at 50 V until the tracking dye entered the separating gel. Later on, the voltage was increased to 85 V. Electrophoresis was carried out until the dye was about 0.5 cm from the bottom of the gel.

#### **3.2.5.6. Staining and de-staining of gels**

Gels were stained overnight with a solution containing 0.1% (w/v) Coomassie Brilliant Blue R-250, 25% methanol and 10% acetic acid. De-staining was done with a solution consisting of 7.5% acetic acid and 10% methanol, until the background was clear.



### 3.2.5.7. Determination of molecular weight of the sub-units

A mixture of molecular weight markers containing carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine plasma albumin (66 kDa), rabbit muscle phosphorylase B (97.4 kDa), *E. coli*  $\beta$ -galactosidase (116 kDa) and rabbit muscle myosin (205 kDa) prepared in a solution containing 1% SDS and 5%  $\beta$ -mercaptoethanol, was boiled for 5 min and run simultaneously along with the samples for estimating the molecular weights of the subunits.

### 3.2.5.8. Identification of High Molecular Weight Glutenin Subunits

High molecular weight glutenin subunits (HMW-GS) which are encoded by genes at the Glu-B1 locus of chromosome 1B were numbered according to the method explained by Payne and Lawrence (1983). Three well characterized known aestivum varieties, namely, DWR 162, GW 322, and Hussar with HMW-GS 7+9, 7+8, and 6+8, respectively, were used as reference samples. Subunit 20 was identified by comparing with a known Italian variety Lira (Masci et al., 1995). Subunit combination 13+16 was identified according to its relative mobility to subunit 20 according to Sreeramulu and Singh (1994) and Boggini et al (1995).

### 3.2.6. Reversed phase – high performance liquid chromatography (RP-HPLC) of gliadins

RP-HPLC of gliadins was done using acetonitrile (CH<sub>3</sub>CN)-water-trifluoroacetic acid (TFA) system as per the procedure explained by Burnouf and Bietz (1984) with some modifications. Durum wheat kernels were ground

with a mortar and pestle and the gliadins were extracted by continuous agitation with ethanol 70% (v/v) (250 mg flour/4 ml 70% ethanol) at room temperature for 30 min. The solution was centrifuged (10 min, 10,000g) and 20  $\mu$ l of the supernatant were chromatographed in RP-HPLC Supelco C18 column (250x4.6 mm; Supelcosil LC-318; bead size 5  $\mu$ ; pore size 300  $\text{Å}$ ) (Bellefonte, PA, USA). Chromatography was carried out using mixtures of two solvents, designated as solvents 'A' and 'B'. Solvent 'A' contained 15% (v/v)  $\text{CH}_3\text{CN}$  and 0.1% (v/v) TFA, and solvent 'B' contained 80% (v/v)  $\text{CH}_3\text{CN}$  and 0.1% (v/v) TFA. The column was equilibrated with a mixture containing 80% solvent 'A' and 20% solvent 'B'. The proteins were eluted using a linear gradient (20% 'B' to 55% 'B') for 55 min, which was initiated upon sample injection. Column temperature was maintained at 31  $^\circ\text{C}$  and the flow rate at 1 ml/min. Protein in the column effluent was monitored continuously by measuring the absorbance at 210 nm. Re-equilibration to initial solvent conditions was achieved in 20 min. Each sample was analyzed in duplicate.

### 3.2.7. Scanning Electron Microscopy (SEM) studies

Scanning electron microscopic studies of different samples were carried out using LEO 435 VP scanning electron microscope (Leo electron microscopy Ltd., Cambridge, UK). Semolina samples were crumbled on to double sticky tape on specimen holder and excess semolina was blown off. The semolina samples were scanned for surface characteristics of individual particles. Raw spaghetti and freeze-dried cooked spaghetti strands were examined both at the surface and in the inner part of the fractured strand with scanning electron microscopy. The samples were mounted on a specimen

holder with carbon coated double sticky tape. Semolina samples and mounted strands were sputtered with gold using Polaron SEM coating system E-5000. Coating time was 2 min and thickness of the coating was 300 Å. The coated samples were loaded on the system and SEM was done at an accelerating voltage of 20 kV using 35mm Ricoh camera.

### **3.2.8. Fractionation of semolina and spaghetti proteins**

A modified Osborne solubility fractionation procedure was applied on semolina and LT- and HT-dried spaghetti from three durum varieties, namely, DWR 2006, MACS 1967, and PDW 274.

Semolina and spaghetti samples were ground to a fine powder using a mortar and pestle. A 3-g sample of ground semolina or spaghetti was extracted stepwise twice (2 h each) with 30 ml of 0.15 M NaCl, three times (2 h, 2 h, 1 h) with 30 ml of ethanol (70%, v/v), and twice (2 h each) with 30 ml of 0.1 M acetic acid solution to give four protein fractions: salt-soluble (albumin and globulin), ethanol-soluble (gliadin), acetic acid-soluble (glutenin), and residue protein, respectively. All extraction steps were performed under refrigerated conditions (4 °C) using a magnetic stirrer. Corresponding supernatants were combined and their protein content was measured by micro-kjeldahl method (AACC, 2000). The protein distribution of each sample was calculated from the volume and protein content of each fraction. The residue fraction was freeze-dried and subjected to protein measurement by micro-kjeldahl method (AACC, 2000).

A small portion of supernatant of each fraction was taken for SDS-PAGE studies. Albumins were separated from globulins by dialysis of the salt extract against distilled water at refrigerated conditions (4 °C). Ethanol was evaporated from ethanol extract using a rotary evaporator (Buchi Laboratoriums – Technik, Flawil/Schweiz, Switzerland). Solutions of albumins, globulins, gliadins, and glutenins were freeze-dried separately and stored in freezer (-20 °C) till using in SDS-PAGE studies.

### **3.2.9. Studies on the effects of microbial transglutaminase (TG) and soy protein isolate (SPI) on semolina and spaghetti properties**

#### **3.2.9.1. Farinograph and pasting properties of semolina**

TG at the levels of 0.5, 1.0, 1.5, and 2.0% (w/w), SPI at the level of 3.0% (w/w), and TG+SPI at two levels of 1%+3% and 2%+3% (w/w) were added to semolina and mixed thoroughly. Farinograph characteristics of control semolina and semolina samples containing different levels of TG, SPI, or TG+SPI were determined at constant water absorption of 35% as described in section 3.2.2.2. Pasting properties of semolina samples were determined according to the method explained in section 3.2.2.3.

#### **3.2.9.2. Production of spaghetti**

Different levels of TG (0.5, 1.0, 1.5, 2.0, 3.0%; w/w), 3.0% (w/w) of SPI, and two levels of TG+SPI (1%+3% and 2%+3%; w/w) were added to semolina and dry mixed thoroughly. Dough preparation and extrusion was carried out

according to the procedure explained in section 3.2.3.1. The extruded spaghetti strands were dried using high temperature (HT) (85 °C) drying method as described in section 3.2.3.1.

Small portions of dough from each sample before and after extrusion were taken for protein solubility and SDS-PAGE studies. For termination of the enzyme reaction, the dough portions were immediately cooled in ice water. Samples were then frozen, freeze-dried and stored in freezer (-20 °C) until analyzed.

#### **3.2.9.3. Protein solubility of spaghetti dough**

Control and TG-treated freeze-dried mixed dough (before extrusion) samples were ground using a mortar and pestle. A 1-g powdered sample was suspended in 10 ml of buffer solution containing phosphate (27.5 mM, pH 7.5), 4% (w/v) SDS, and 5% (v/v)  $\beta$ -mercaptoethanol and left for 30 min extraction with vortex mixing every 5 min. The suspension was centrifuged at 8,000g in a refrigerated centrifuge for 10 min. The supernatant was decanted and the residue fraction was freeze-dried. Protein content of supernatant and residue samples was determined by micro-kjeldahl method (AACC, 2000).

#### **3.2.9.4. SDS-PAGE**

Freeze-dried mixed dough and extruded dough samples (control and treated with 0.5, 1.0, 1.5, and 2.0% w/w TG) were powdered using a mortar and pestle, and 25 mg of each sample was dissolved in 400  $\mu$ l of extraction buffer solution. SDS-PAGE was carried out on a vertical 10% polyacrylamide gel as described in section 3.2.4.

### **3.2.9.5. Evaluation of spaghetti quality**

Quality characteristics of TG, SPI, and TG+SPI treated dry spaghetti and cooking quality characteristics of corresponding spaghetti samples were determined according to the procedures described in sections 3.2.3.2 and 3.2.3.3, respectively.

### **3.2.10. Studies on effects of microbial lipase and distilled glycerol monostearate (DGMS) on semolina and spaghetti properties**

#### **3.2.10.1. Pasting properties of semolina**

To study the effect of different levels of lipase (25, 50, 100, 150, and 200 ppm) on semolina pasting properties, a stock solution (250 ppm) was prepared by dissolving 25 mg of lipase in 100 ml of distilled water. The solution was freshly prepared for each set of experiment and kept in refrigerator throughout the experiment period. Different volumes of 1.5, 3.0, 6.0, 9.0, and 12.0 ml corresponding to 25, 50, 100, 150, and 200 ppm of enzyme were transferred to the water used for suspending the powdered semolina samples. Above mentioned volumes had been deducted from the calculated amount of water required for conducting the amylograph test for each sample. DGMS at the level of 0.5% (w/w) and combination of 0.5% and 50 ppm of lipase was added to the powdered semolina and mixed thoroughly. Pasting properties of different samples were determined according to the procedure explained in section 3.2.2.3.

**3.2.10.2. Spaghetti production and quality evaluation of spaghetti**

Different levels of lipase (50, 100, 150 ppm), 0.5% (w/w) DGMS, and a combination of '50 ppm of lipase + 0.5% of DGMS' were dissolved in the water used for dough preparation. Dough preparation and extrusion, and spaghetti drying (high temperature) was carried out according to the methods described earlier in section 3.2.3.1. Quality characteristics of dry and cooked spaghetti samples were determined as per the procedures described in sections 3.2.3.2 and 3.2.3.3, respectively.

**3.2.11. Statistical analysis**

The data were statistically analyzed using Duncan's New Multiple Range Test (Duncan, 1955). Data were mean of at least three determinations unless otherwise mentioned. Correlation coefficients were determined using Microsoft Excel 2000 software.

### 4.1A.1. Introduction

Durum wheat (*Triticum durum*) is known as the most suitable raw material for the production of pasta products such as spaghetti, macaroni, and vermicelli. This is mainly because of its relatively high yellow pigment content, low lipoxygenase activity and high protein content. According to Feillet (1988) the average protein content of durum wheat is higher than that of common wheat and its protein may vary from 9 -18%. There is a general agreement that protein content of durum wheat is known as an important primary factor influencing pasta quality and the quality of this protein is a secondary factor (Matsuo and Irvine, 1970; Matsuo et al., 1972; Dexter and Matsuo, 1977b; Grzybowski and Donnelly, 1979; Dexter and Matsuo, 1980; Del Nobile et al., 2005). The ability of proteins to form an insoluble network able to entrap the swollen and gelatinized starch granules and to avoid disruption of the spaghetti surface and leaching of carbohydrates and proteins by the boiling water forms the basis of good cooking quality in durum wheat (Feillet, 1988). On the other hand, during cooking, a weak or discontinuous protein matrix results in a protein network that is too loose and permits a greater amount of exudates to escape during starch gelatinization (Resmini and Pagani, 1983). Therefore, higher the protein content, the polypeptide chains are more numerous and the chances for proteins to interact and to form a resistant network are higher (Feillet, 1988).

The suitability of a wheat cultivar for different end uses, viz. bread, chapatti, biscuit, noodle and pasta products is determined to a large extent by its seed protein composition (MacRitchie, 1992). Presence of certain glutenin



subunits have shown to be associated with superior rheological properties of gluten, such as elasticity and dough strength (Pogna et al., 1990; Carrillo et al., 1990; Shewry et al., 1994). Association between the presence of certain high molecular weight glutenin subunits (HMW-GS) and bread making quality is well documented (Payne et al., 1987; Kolster and Vereijken, 1993). Association between HMW-GS and gluten quality has been also found in durum wheat (Boggini and Pogna, 1989). In durum wheat, the most important HMW-GS are encoded by genes at the Glu-B1 locus on the long arm of chromosome 1B. These subunits are useful not only for the identification of wheat cultivars, but can also be used by breeders in choosing parental lines with superior gluten quality (Sreeramulu and Singh, 1994).

Yellow pigments are a major quality factor in durum wheat endosperm and are often referred to as carotenoids, which could include both carotene and the xanthophylls (Irvine, 1971; Youngs, 1988). Traditionally, yellow pasta is considered a mark of quality, mainly because yellow color is closely associated with durum wheat which generally gives pasta with superior cooking quality. Therefore, apart from protein content, yellow pigments content of durum wheat can also be used as another primary factor for initial screening of wheat.

However, it should be noted that a high level of yellow pigments does not always result in a high yellow color of pasta because the yellow pigment content of durum seed can be affected by storage of grain (Dahle, 1965) or semolina, and the milling process (Chen and Geddes, 1945; Borrelli, et al.,

1999), or the oxidative degradation of pigments by lipoxygenase (LOX) enzymes during pasta processing (Irvine and Winkler, 1950; Irvine and Anderson, 1953).

Apart from LOX which influences the yellow pigment content of pasta, peroxidase (POD) and polyphenol oxidase (PPO) enzymes are known to affect the yellowness of pasta. POD is not specific in its reactions and catalyzes the oxidation of a large number of phenols and aromatic rings which occur naturally in plant tissues (Feillet et al., 2000). Kobrehel et al (1972), and Taha and Sagi (1987) found a strong correlation between pasta brownness and POD activity. PPO catalyses the oxidation of phenolic compounds in the presence of molecular oxygen (Feillet et al., 2000). Numerous studies have indicated the deleterious effect of high levels of PPO on aestivum wheat products such as chapatti (Abrol and Uprety, 1970; Singh and Sheoran, 1972) and oriental noodles (Kruger et al., 1994; Baik et al., 1995). Several workers have also shown the role of PPO in pasta brownness (Menger, et al., 1969; Kobrehel et al., 1972).

Besides the above mentioned enzymes which are important for color of pasta, proteases are another enzyme system which is known to be technologically important with regard to cooking quality of pasta. However, Feillet (1988) pointed out that proteolytic enzymes probably have no effect on the quality of pasta made from sound wheat but that highly sprouted wheats may have a negative influence by supplying endoproteolytic activities.

In the present chapter, results of screening of fourteen Indian durum varieties procured from various Indian Agricultural Universities and wheat breeding centers are presented. Since chemical characteristics of these varieties with respect to their protein, yellow pigment content and enzyme activities were not known, representative portions of each variety was processed into whole wheat flour and were analyzed for their total protein, yellow pigments as well as for the activities of enzymes namely, LOX, POD, PPO and protease, which are directly or indirectly related to the pasta making properties of the durum wheat. For comparative studies, two Indian aestivum wheat varieties namely, HD 2189 and HD 2781 were also analyzed along with the durum varieties. Since there is limited information on the allelic variation of HMW-GS among the Indian durum wheat cultivars, distribution of HMW-GS in the 14 Indian durum wheat varieties was also determined and the results reported in this chapter.

#### **4.1A.2. Proximate composition of wheat varieties**

Moisture, ash, and protein contents of whole wheat flours of 14 durum and 2 aestivum wheat varieties are shown in Table 5. Moisture content in durum wheat samples varied from 9.0% to 11.5%. The moisture content in the two aestivum wheat varieties was marginally lower. The ash content varied between 1.38% and 2.14% in durum samples and in the aestivum varieties it was within the above range. The protein content among durum varieties varied between 10.7% (WH 896) and 15.9% (MACS 1967). Durum wheats are known to have higher protein content compared to aestivum wheats

Table 5. Proximate composition of wheat varieties

Variety	Moisture (%)	Ash * (%)	Total Protein * (Nx5.7) (%)
<b>DWR 2006</b>	10.95 ±0.02 <sup>b</sup>	1.96 ±0.03 <sup>cd</sup>	14.19 ± 0.41 <sup>de</sup>
<b>HI 8498</b>	9.0 ±0.07 <sup>f</sup>	1.38 ±0.05 <sup>e</sup>	13.82 ± 0.14 <sup>ef</sup>
<b>MACS 1967</b>	10.50 ±0.18 <sup>c</sup>	1.96 ±0.04 <sup>cd</sup>	15.92 ± 0.28 <sup>abc</sup>
<b>MACS 2694</b>	11.40 ±0.26 <sup>a</sup>	1.98 ±0.05 <sup>cd</sup>	13.65 ± 0.25 <sup>efg</sup>
<b>MACS 2846</b>	9.85 ±0.2 <sup>de</sup>	2.05 ±0.0 <sup>abc</sup>	15.03 ± 0.08 <sup>cd</sup>
<b>MACS 3125</b>	9.90 ±0.13 <sup>de</sup>	2.14 ±0.03 <sup>a</sup>	15.31 ± 0.0 <sup>bc</sup>
<b>N 59</b>	9.75 ±0.04 <sup>e</sup>	2.04 ±0.01 <sup>abc</sup>	15.55 ± 0.10 <sup>bc</sup>
<b>NIDW 15</b>	10.10 ±0.08 <sup>d</sup>	1.92 ±0.02 <sup>d</sup>	13.13 ± 0.18 <sup>fg</sup>
<b>NIDW 295</b>	9.95 ±0.05 <sup>de</sup>	2.03 ±0.0 <sup>bcd</sup>	12.76 ± 0.33 <sup>gh</sup>
<b>PDW 215</b>	10.80 ±0.12 <sup>b</sup>	1.93 ±0.04 <sup>cd</sup>	12.70 ±0.15 <sup>gh</sup>
<b>PDW 233</b>	11.55 ±0.01 <sup>a</sup>	1.95 ±0.03 <sup>cd</sup>	12.10 ± 0.21 <sup>h</sup>
<b>PDW 274</b>	10.55 ±0.08 <sup>c</sup>	1.94 ±0.03 <sup>cd</sup>	13.07 ± 0.05 <sup>fg</sup>
<b>Raj 1555</b>	10.15 ±0.11 <sup>d</sup>	2.12 ±0.01 <sup>ab</sup>	12.90 ± 0.28 <sup>g</sup>
<b>WH 896</b>	10.20 ±0.10 <sup>d</sup>	1.92 ±0.03 <sup>d</sup>	10.70 ±0.21 <sup>i</sup>
<b>Range</b>	9.00 – 11.55	1.38 – 2.14	10.70 – 15.92
<b>Mean</b>	10.13	1.95	13.63
<b>HD 2189**</b>	8.85 ±0.07 <sup>fg</sup>	1.91 ±0.0 <sup>d</sup>	15.95 ± 0.03 <sup>ab</sup>
<b>HD 2781**</b>	8.65 ±0.09 <sup>g</sup>	2.15 ±0.02 <sup>a</sup>	16.57 ± 0.16 <sup>a</sup>

\*Expressed on dry basis.

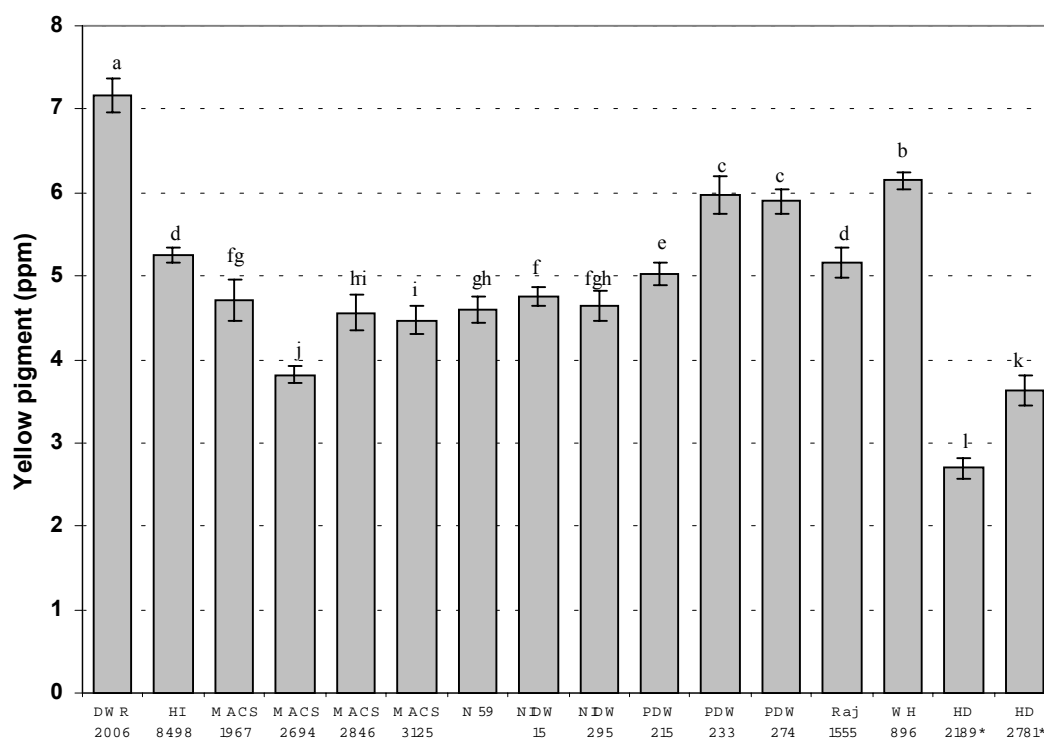
\*\*Aestivum varieties.

Data are expressed as mean ± SD. Means of same column followed by different letters differ significantly ( $p < 0.05$ ).

(Halversan and Zeleny, 1988). Analysis of the two aestivum wheat showed that they had protein contents of 15.9% and 16.6% respectively. The protein content in the two aestivum varieties was either at par or more than that found in the 14 durum varieties analyzed. Matveef (1966) showed that there is a very consistent relationship between the protein content of wheat and the cooking quality of pasta. Accordingly, a wheat protein content higher than 13% was reported to yield a satisfactory final product, whereas protein content lower than 11% gave a poor product. Wheat protein content appears to account for 30-40% of the variability in cooking quality of pasta (Dexter et al., 1980). It has earlier been reported that protein content in Indian aestivum wheats is relatively lower compared to durum varieties (Ranga Rao et al., 1981). However, in our study we found that the aestivum wheats had relatively high protein content.

#### **4.1A.3. Yellow pigment content**

Among the 14 durum varieties, DWR 2006 had significantly highest yellow pigment content (7.2 ppm) followed by the varieties WH 896 and PDW 233 which had pigment content of 6.15 ppm and 6.0 ppm respectively (Fig. 23). These values are comparable with those of two Canadian durum wheats, which had pigment content of 5.5 and 6.5 ppm, respectively (Dexter and Matsuo, 1978a). Still higher pigment values of 9.93 and 7.54 ppm were reported in two Indian durum wheat varieties by Kathuria and Sidhu (1984a). Dexter et al (1994) had reported similar values of 7.2 and 9.0 ppm in two Canadian durum wheat varieties. Except for the variety MACS 2694, yellow pigment content in other durum varieties were between 4.5 and 5.3 ppm.



**Fig. 23.** Yellow pigment content in different wheat varieties (\*aestivum varieties). Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

These values compare well with 25 Italian durum cultivars which had yellow pigment between 3.0 and 6.5 ppm (Borrelli et al., 1999). MACS 2694 was the only variety among the 14 durum tested to have significantly the lowest yellow pigment content of 3.8 ppm. Haridas Rao et al (1976) studied twenty four Indian durum wheat varieties in which the yellow pigment content ranged from 2.72 to 6.47 ppm which was relatively lower than some of the newly developed varieties reported here. Unlike the protein content which was equally high in the two aestivum wheat varieties studied (HD 2189 and HD 2781); the yellow pigment content was found to be significantly low at 2.7 ppm and 3.6 ppm, respectively. These values were comparable with the durum variety MACS 2694. Durum wheats and semolina generally contain much more of yellow pigments than bread wheats and flours (Laignelet, 1983).

Many studies have confirmed the genetic variability and high heritability of yellow pigment content in durum wheat varieties (Irvine and Anderson, 1953; Matz and Larsen, 1954; Borrelli et al., 1999). The yellow pigments are not homogeneously distributed in the wheat kernel; with the embryo, bran, and endosperm containing decreasing levels of carotenoids (Quaglia, 1988). Borrelli et al (1999) found that carotenoids in some durum varieties are distributed in the inner kernel layers, and then pigment loss during milling of these kinds of varieties to semolina will be less. Accordingly, they pointed out that high pigment content in pasta products is not always derived from higher pigment content in whole grain.

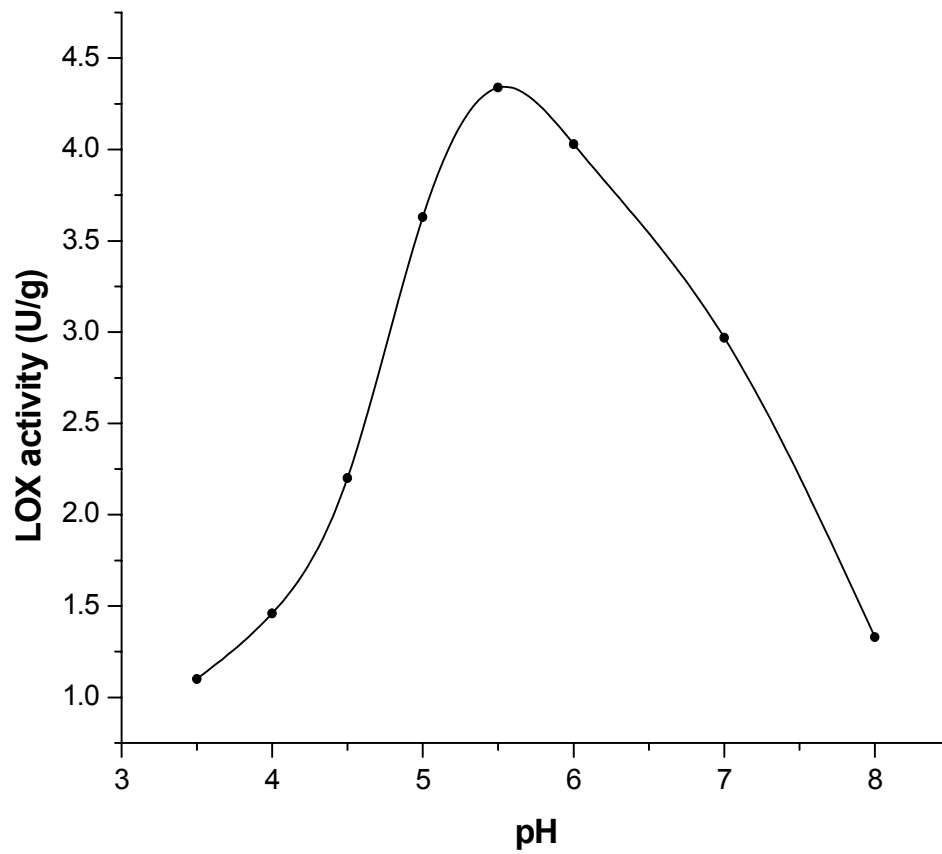
#### 4.1A.4. Lipoxygenase activity

To find out the pH optima for LOX activity, the enzyme extracts from 14 durum varieties were assayed in buffers ranging in their pH values from 3.5 to 8.0. All wheat varieties showed maximum LOX activity at pH 5.5. Fig. 24 shows a typical graph for LOX activity at different pH values and optimum pH for durum variety PDW 233.

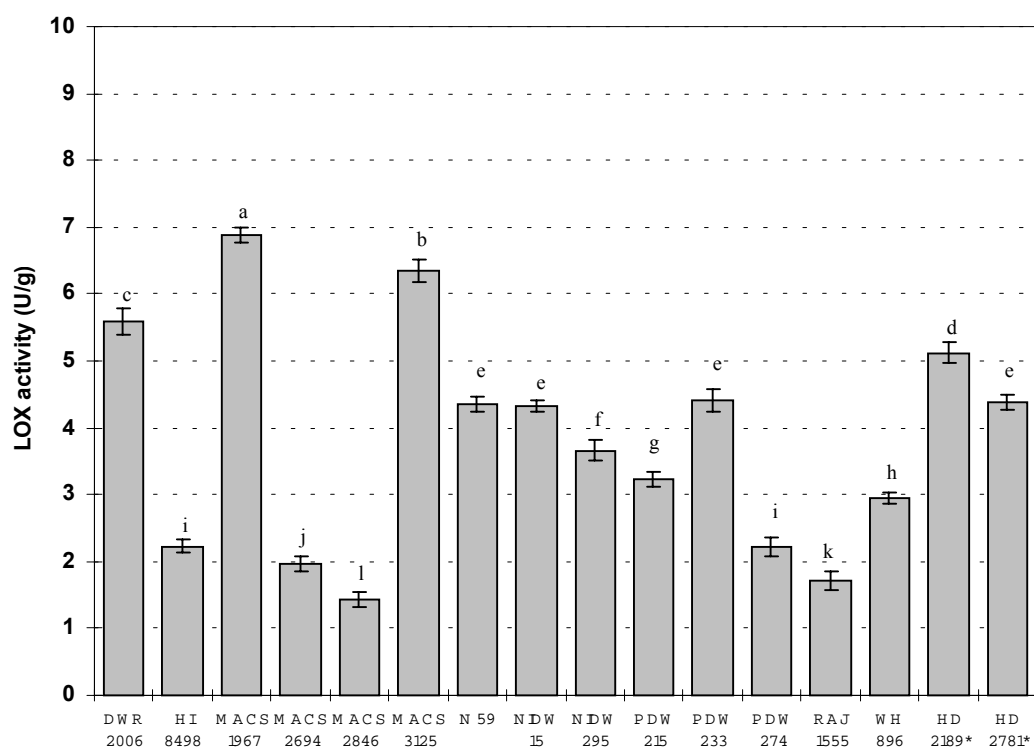
LOX levels in durum wheat are cultivar-related and also environmentally dependent (Irvine and Anderson, 1953; Lee et al., 1976; Borrelli et al., 1999). In the present study, LOX varied widely among the Indian durum wheats. The highest LOX activity was seen in MACS 1967 (6.9 U/g) followed by MACS 3125 (6.3 U/g) (Fig. 25). The durum variety MACS 2846 had significantly lowest LOX activity (1.4 U/g). In the other samples it varied from 1.7 U/g (Raj 1555) to 5.6 U/g (DWR 2006). LOX activity in the two aestivum wheats was also within the above range. Out of the 14 durum varieties analyzed, 8 varieties showed significantly lower LOX activity than the two aestivum varieties. Irvine and Anderson (1953) working on seven Canadian durum varieties found that durum varieties having best macaroni making properties are characterized by low LOX activity; they reported lower LOX activity in some of these varieties that ranged from 10-16  $\mu\text{l O}_2/\text{min/g}$ . Borrelli et al (1999) reported average LOX activity of 4.3 and 5.3 U/g for two isoenzymes of LOX, respectively, in some Italian cultivars.

As discussed earlier, variety DWR 2006 had significantly high amount of pigment content. This variety also had relatively high LOX activity. Borrelli et al (1999) pointed out that the desirable yellow color in pasta products is not





**Fig. 24.** Typical graph for Lipoxigenase (LOX) activity of durum wheat variety PDW 233 as a function of pH



**Fig. 25.** Lipoxigenase (LOX) activity in different wheat varieties (\*aestivum varieties). Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

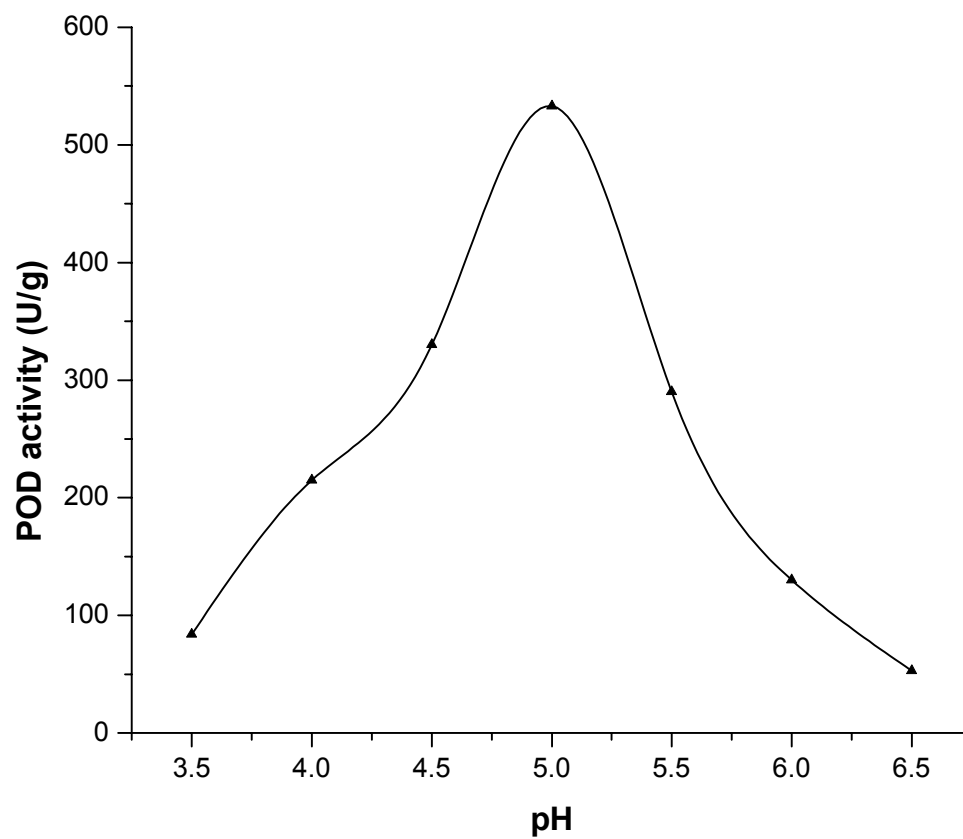
always derived from higher pigment content but rather lower LOX activities. However, Auerman et al (1971) reported that wheat germ and bran contain 17 fold and 4 fold more LOX activity, respectively, than the endosperm. It can however be noted here that during milling of wheat into semolina most of the bran and germ is removed. Rani et al (2001) showed that an aestivum variety which had LOX activity of 1.5 U/g reduced to about 0.5 U/g during milling. In their study semolina was one of the flour mill streams containing significantly lower LOX activity than most of the other flour mill streams.

Taking into consideration both the LOX activity and the pigment contents, it can be concluded that among the durum varieties studied, perhaps the variety DWR 2006, WH 896, and PDW 233 with their relatively high pigment content and MACS 2846, Raj 1555, PDW 274 and HI 8498 with their relatively lower LOX activities and higher yellow pigment content would be more suitable for spaghetti preparation as far as the color of the product is considered. In comparison, the two aestivum wheats tested had relatively high LOX activity and lower pigment contents, hence would be less suitable for spaghetti preparation with respect to the color of product. Though varieties MACS 1967, MACS 3125 and DWR 2006 have high LOX activity, but since they have sufficient amount of protein and yellow pigment contents, they can perhaps be considered as good varieties for pasta making. It is known that many compounds which act as physiological antioxidants such as  $\beta$ -carotene (Lomnitski et al., 1993), and also  $\alpha$ -tocopherol and L-ascorbate (Buettner, 1993) are able to inhibit the LOX from various systems. Similarly, it has been found that  $\beta$ -carotene (Trono et al., 1999),  $\alpha$ -tocopherol (Pastore et al., 2000)

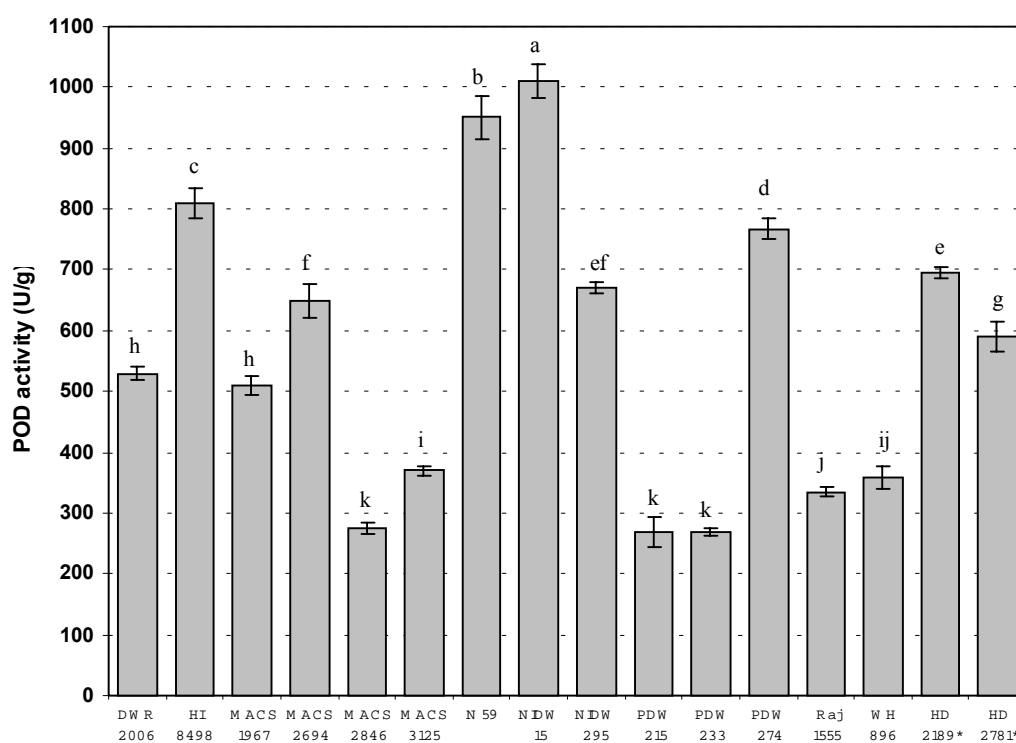
and L-ascorbate (McDonald, 1979; Pastore et al., 2000) inhibit  $\beta$ -carotene bleaching in durum wheat semolina. Kathuria and Sidhu (1984a,b) showed that LOX activity in durum wheat can be inhibited by using hot water conditioning of wheat for milling into semolina. However, since LOX is genetically controlled, the induction of lines lacking in one or more LOX isoenzymes, which can be employed in breeding programs, could be useful in reducing LOX levels in durum wheat cultivars (Borrelli et al., 1999).

#### 4.1A.5. Peroxidase activity

POD activity assay for different wheat varieties at different pH values showed that the maximum activity of this enzyme was at pH 5.0. Fig. 26 shows the POD activity of durum variety DWR 2006 as a representative of all wheat varieties. Presence of POD among the 14 durum varieties showed wide variation (Fig. 27). The highest value was seen in the variety NIDW 15 with a POD activity of 1010 U/g followed by the variety N 59 (950 U/g). Lowest POD activity of 269 U/g was seen in the durum variety PDW 233. Second lowest value (270 U/g) was found in PDW 215, but there was no significant difference between these two varieties and MACS 2846 in terms of POD activity. Amongst the other samples, HI 8498 and PDW 274 had POD values above 700 U/g. Fraignier et al (2000) also found a large variability in POD activity between durum wheat cultivars. Aestivum variety HD 2781 had a value of 590.4 U/g while HD 2189 showed a value of 695.6 U/g, which were higher than the average POD activity for 14 durum varieties. Earlier Rani et al (2001) reported a value of 310 U/g in an Indian aestivum wheat variety with the bran fractions having the most activity. They also found that POD activity



**Fig. 26.** Typical graph for Peroxidase (POD) activity of durum wheat variety DWR 2006 as a function of pH

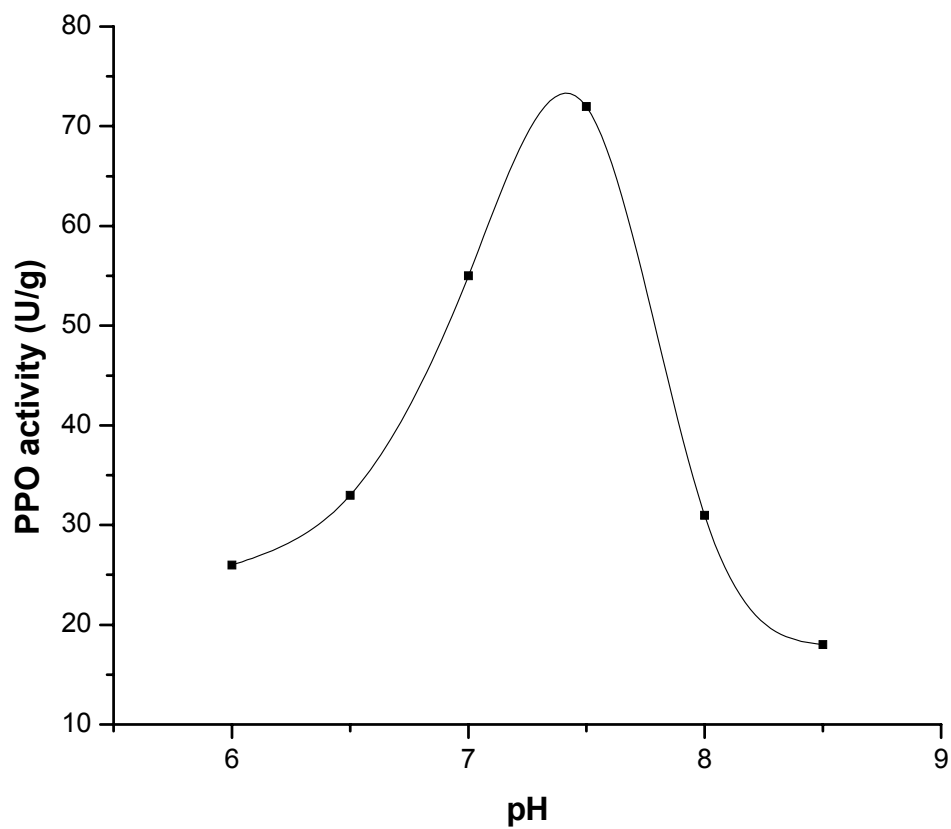


**Fig. 27.** Peroxidase (POD) activity in different wheat varieties (\*aestivum varieties). Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

in semolina milled from the same wheat was about five fold less than that found in whole wheat flour. Outer layers of the endosperm are generally very rich in POD activity (Honold and Stahmann, 1968). Hence, it is preferable to eliminate most of it during milling. Pasta products made from cultivars with a high POD activity develop an undesirable brownish color during processing (Fraignier et al., 2000). Kobrehel et al (1972) also found that POD activity was positively correlated to brown index of pasta products.

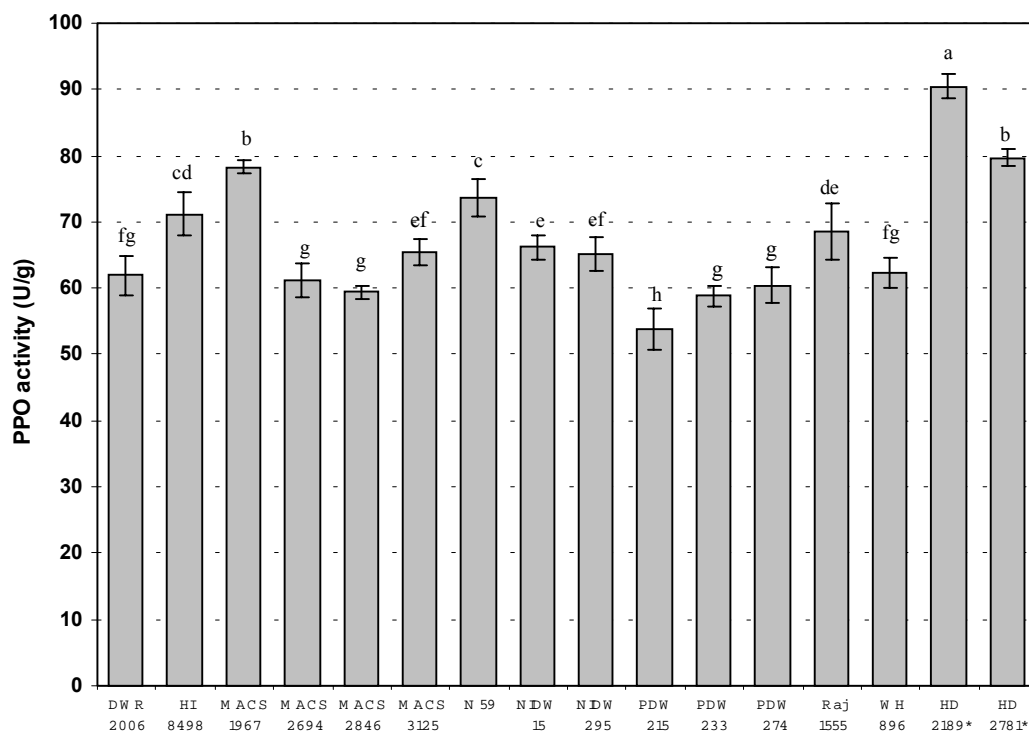
#### **4.1A.6. Polyphenol oxidase activity**

In examining the activity of PPO enzyme from different varieties at different pH values, it was observed that this enzyme had no significant activity in acidic pH and maximum activity was achieved at pH 7.5. As an example, PPO activity of durum variety N 59 was plotted against different pH values ranging from 6.0 to 8.5 (Fig. 28). PPO activity in wheat is cultivar – related and is also affected by growing conditions (Baik et al., 1994). Fig. 29 shows the PPO activity in the durum and aestivum wheats. In the durum varieties, PPO activity varied from 53.8 (PDW 215) to 78.3 (MACS 1967) U/g. Unlike the POD activity where lot of variation was observed among the samples, PPO activity did not vary much among different varieties. Amongst the durum and aestivum varieties, aestivum variety HD 2189 had significantly the highest PPO activity followed by aestivum variety HD 2781. However, there was no significant difference between PPO activity of aestivum variety HD 2781 and durum variety MACS 1967. The two Indian aestivum varieties had higher PPO activities than durum varieties. Lamkin et al (1981) also found



**Fig. 28.** Typical graph for Polyphenol oxidase (PPO) activity of durum wheat variety N 59 as a function of pH



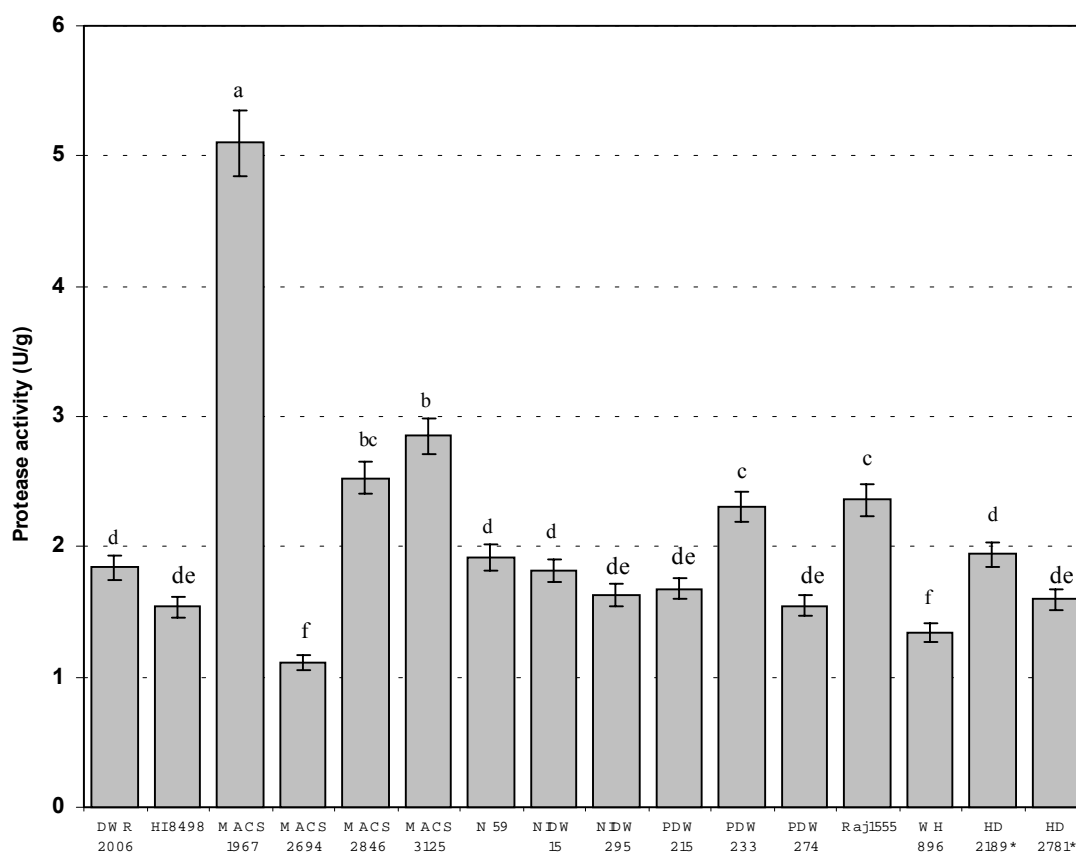


**Fig. 29.** Polyphenol oxidase (PPO) activity in different wheat varieties (\*aestivum varieties). Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

that durum wheats had significantly lower PPO activity than the other classes of wheat. Feillet and Kobrehel (1974) found that certain PPO was present in hexaploid but not in tetraploid wheats, which can be used as a marker to determine common wheat present as an adulterant in semolina and pasta products. Kobrehel et al (1974) have explained that along with POD, PPO activity would control the brownness of pasta product. Taha and Sagi (1987) also confirmed the role of semolina PPO activity on pasta browning. However, Dexter et al (1994) working on debranned and non-debranned wheats have explained that the low level of PPO found in processed wheats makes it unlikely that PPO activity is a major factor determining spaghetti color. Similar to POD, PPO is concentrated in the branny layers of wheat (Marsh and Galliard, 1986; Hatcher and Kruger, 1993). Therefore, a considerable part of this enzyme can be removed during durum wheat milling and semolina production, followed by a well done purification of semolina (Hatcher and Kruger, 1993). Rani et al (2001) reported PPO activity of 40 U/g in whole wheat flour of an Indian aestivum variety. In their study, the highest values of PPO were recorded in the bran fractions, while PPO activity in farina was very low.

#### **4.1A.7. Protease activity**

Results of protease activity are shown in Fig. 30. In the present study, MACS 1967 had significantly highest protease activity (5.1 U/g). The protease activity in other durum varieties was significantly much lower than that of MACS 1967. MACS 2694 with protease activity of 1.1 U/g had the lowest level of activity. Generally, there was not much variation in the protease activity of durum and aestivum varieties that ranged from 1.55 to 2.85 U/g, except for



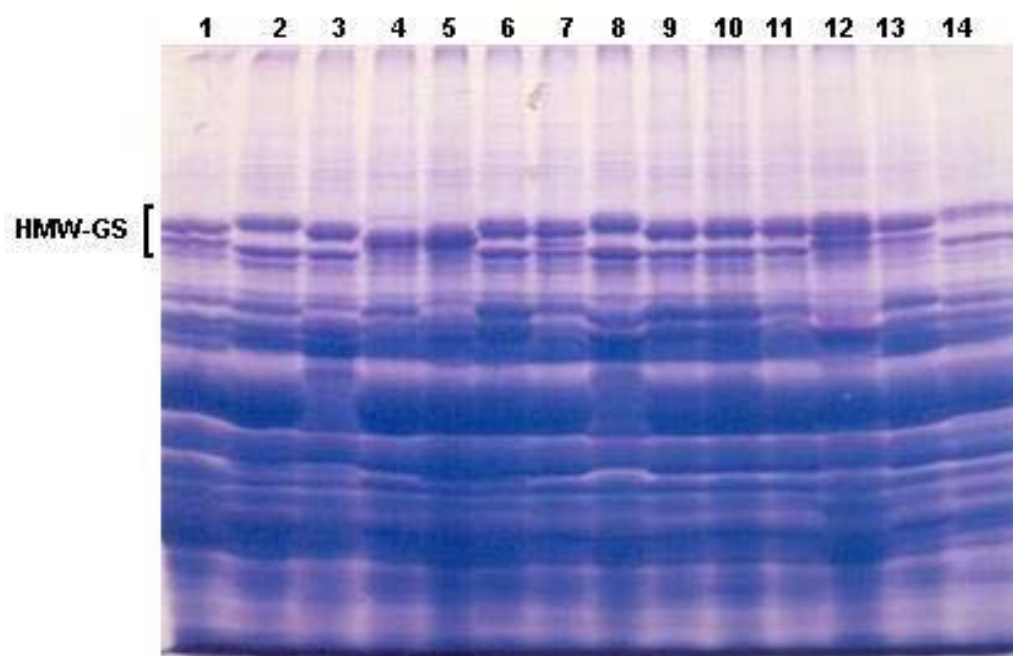
**Fig. 30.** Protease activity in different wheat varieties (\*aestivum varieties). Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

MACS 1967 and MACS 2694. Feillet (1988) pointed out that the durum wheat proteases have no effect in determining the quality of pasta processed from sound wheat. However, proteolytic enzymes affecting the ability of semolina to produce high quality spaghetti by enzymatic breakdown of only a few peptide bonds cannot be overruled (Petruzzelli et al., 1981).

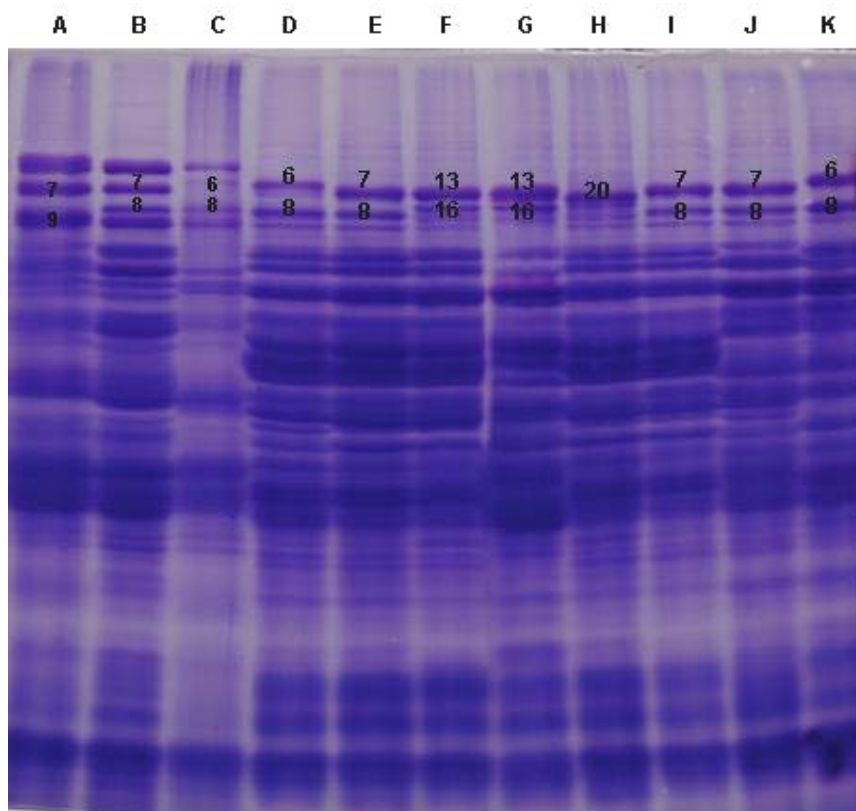
#### **4.1A.8. Variation in the high molecular weight glutenin subunits**

High molecular weight glutenin subunit (HMW-GS) composition of whole wheat flours from 14 Indian durum wheat varieties was determined using SDS-PAGE analysis on 10% gel. Fig. 31 shows SDS-PAGE fractionation of total endosperm protein from the 14 varieties. Representative varieties for HMW-GS composition along with wheat cultivars with known subunits were fractionated on 10% gel SDS-PAGE to identify the HMW-GS allelic composition (Fig. 32). Amongst the 14 Indian durum varieties examined, four alleles were identified (subunits 6+8, 7+8, 13+16 and 20) which belong to Glu-B1 locus on the chromosome 1B. The allelic composition at the Glu-B1 locus of each of the 14 varieties is shown in Table 6. Subunit 7+8 was observed more frequently than other subunits. Subunits 6+8 and 13+16 had the second highest frequency followed by subunit 20.

Several attempts have been made by earlier workers to correlate durum wheat gluten strength with HMW-GS. Liu et al (1996) found a positive correlation between HMW subunits 13+16 and 7+8 and gluten strength, while subunit 20 showed a negative correlation. Pogna et al (1990) also reported that HMW subunits 7+8 are associated with large SDS sedimentation volumes



**Fig. 31.** SDS-PAGE fractionation of total endosperm proteins of (1) MACS 1967, (2) DWR 2006, (3) PDW 274, (4) PDW 215, (5) MACS 2846, (6) HI 8498, (7) MACS 3125, (8) NIDW 15, (9) NIDW 295, (10) Raj 1555, (11) PDW 233, (12) N 59, (13) MACS 2694, (14) WH 896.



**Fig. 32.** SDS-PAGE fractionation of total endosperm proteins of (A) DWR 162, (B) GW 322, (C) Hussar, (D) DWR 2006, (E) MACS 3125, (F) MACS 2694, (G) N 59, (H) PDW 215, (I) PDW 233, (J) PDW 274, (K) NIDW 15.

**Table 6.** High molecular weight glutenin subunit composition of 14 Indian durum wheat varieties

<b>Variety</b>	<b>HMW-GS on Glu-B1 locus</b>
<b>DWR 2006</b>	6+8
<b>HI 8498</b>	7+8
<b>MACS 1967</b>	13+16
<b>MACS 2694</b>	13+16
<b>MACS 2846</b>	20
<b>MACS 3125</b>	7+8
<b>N 59</b>	13+16
<b>NIDW 15</b>	6+8
<b>NIDW 295</b>	7+8
<b>PDW 215</b>	20
<b>PDW 233</b>	7+8
<b>PDW 274</b>	7+8
<b>Raj 1555</b>	7+8
<b>WH 896</b>	6+8

compared with subunits 6+8 or 20. Kovacs et al (1993) found that breeding lines containing HMW subunits 6+8 had significantly higher SDS sedimentation volumes than those containing HMW glutenin subunit 20. However, they observed that SDS sedimentation volume test could not be a good predictor of pasta quality. On the other hand, it is reported that HMW-GS 6+8 or 7+8 give better pasta quality than HMW subunit 20 (Kovacs et al., 1995). However, Matsuo and Irvine (1970) have pointed out that although gluten strength is a good indicator of dough quality, it may not guarantee a good cooking quality in pasta. Hence, the relationship between the cooking quality of pasta and the occurrence of some specific HMW-GS is still controversial (Feillet, 1988).

#### **4.1A.9. Conclusions**

Protein, yellow pigment and enzymes present in durum wheat are important, in that, they influence the quality of the pasta products for which it is used. From the above studies, some important observations made were, the durum variety DWR 2006 had significantly the highest level of yellow pigment content but its LOX activity was also on the higher side. This variety also had high protein content. MACS 1967 had the highest amount of protein amongst the 14 varieties tested, it had significant amount of yellow pigment content, but it also had the highest LOX activity. The lowest POD activity and the second highest pigment content were observed in durum variety PDW 233, but this variety had significantly lowest amount of protein content among the varieties. The durum varieties HI 8498 and MACS 2846 had high protein content, low LOX activity and significant amount of yellow pigments. MACS



2846 had the lowest level of LOX, and also low level of POD and PPO activities. On the other hand, varieties HI 8498, MACS 3125, NIDW 295, PDW 233, PDW 274 and Raj 1555 had HMW glutenin subunits 7+8 which is reported to have a positive effect on the protein quality.

Based on the results of this chapter, six durum varieties, namely, DWR 2006, MACS 1967, MACS 2694, PDW 215, PDW 274, and WH 896 were selected for further work on their suitability for spaghetti production. DWR 2006 was selected for its high yellow pigment, MACS 1967 was selected because of its high protein content. MACS 2694 had the lowest content of yellow pigment, but it showed very low LOX activity. PDW 215 had HMW-GS 20, medium amount of protein and considerable amount of yellow pigment. PDW 274 was a representative of varieties having HMW-GS 7+8. These six durum varieties were milled into semolina and used for spaghetti making studies. Results of these studies are discussed in the second chapter.

### 4.1B.1. Introduction

The first and perhaps the most important step in durum wheat quality evaluation is development and standardization of a laboratory scale milling procedure which yields semolina of considerable extraction and desirable quality for pasta production. To mill durum wheat into semolina, four basic steps including wheat cleaning, tempering, milling, and purifying are necessary. The process of durum milling, which is a complex procedure of repetitive grinding and sieving, and also semolina purification are the two main steps which completely differentiates it from common wheat milling. Milling performance of durum wheat is evaluated by semolina yield, appearance, and granulation (Dick and Youngs, 1988).

The Buhler laboratory mill is a standard equipment for evaluating wheat and flour at a laboratory scale. Use of a Buhler laboratory mill for semolina milling was first introduced by Black and Bushuk (1967) with a number of minor modifications. Milling conditions for semolina production could be optimized by making certain adjustments to roll speed, gap and differential while the mill is in operation (Manthey and Hareland, 2001). The Buhler laboratory mill (MLU 202) works under a constant differential (2:1) and speed (500:250 rpm). However, by proper gap adjustments along with optimum tempering time and moisture addition, semolina of maximum yield, desirable appearance and particle size could be achieved.

In the present chapter a Buhler laboratory mill (MLU 202) was employed in milling of semolina. A series of trial milling experiments were

carried out to optimize the conditioning moisture, tempering time, and roll gap adjustments prior to the final semolina milling. Durum variety MACS 2694 was used in the milling standardization studies.

#### **4.1B.2. Effect of break-roll gap settings on semolina milling properties**

The Buhler laboratory mill (MLU 202) employed in milling of semolina had three sets of break rolls and three sets of reduction rolls, respectively. For semolina milling only break rolls were used and the reduction rolls were kept idle. In the present experiment, the roll gap between the pair of first break (B1) was adjusted from 0.3 mm to 0.85 mm and the gap between the pair of third break rolls (B3) was kept constant at 0.2 mm. Adjusting the gap between the pair of third break rolls at either 0.15 mm or 0.25 mm produced semolina which was found unacceptable in terms of appearance and particle size. The conditioning time was kept constant at 18 h and the conditioning moisture at 16.5%. Several workers have reported that conditioning time of 18 h and conditioning moisture of 16.5% to be suitable for durum wheat milling (Dexter and Matsuo, 1978a; Dexter et al., 1982; Donnelly and Ponte, 2000).

Results of break-roll gap adjustments on semolina yield and on semolina particle size distribution are shown in Tables 7 and 8, respectively. Values shown in Table 7 are those for unpurified semolina. The result shows that as the gap setting increased from 0.3 mm to 0.85 mm, the semolina yield decreased and as a consequence the bran yield, contaminated with coarse

**Table 7.** Effect of different gap adjustments of break rolls on semolina milling properties\*

Break roll gaps (mm)		Milling fractions (%)		
B1	B3	Semolina	Flour	Bran
0.30	0.2	76.6 ±0.31 <sup>a</sup>	5.7 ±0.12 <sup>a</sup>	17.6 ±0.20 <sup>f</sup>
0.35	0.2	74.3 ±0.25 <sup>b</sup>	5.6 ±0.19 <sup>a</sup>	20.0 ±0.15 <sup>e</sup>
0.40	0.2	74.2 ±0.15 <sup>b</sup>	4.2 ±0.25 <sup>c</sup>	21.5 ±0.11 <sup>d</sup>
0.45	0.2	73.2 ±0.30 <sup>c</sup>	4.7 ±0.15 <sup>b</sup>	21.6 ±0.31 <sup>d</sup>
0.50	0.2	66.4 ±0.25 <sup>d</sup>	2.9 ±0.25 <sup>d</sup>	30.7 ±0.17 <sup>c</sup>
0.70	0.2	66.0 ±0.33 <sup>d</sup>	3.0 ±0.30 <sup>d</sup>	30.6 ±0.12 <sup>c</sup>
0.80	0.2	59.0 ±0.18 <sup>e</sup>	2.8 ±0.28 <sup>d</sup>	38.2 ±0.25 <sup>b</sup>
0.85	0.2	58.0 ±0.28 <sup>f</sup>	2.3 ±0.17 <sup>e</sup>	39.7 ±0.18 <sup>a</sup>

\*Conditioning time of 18 h and conditioning moisture of 16.5%

Data are expressed as mean ± SD. Means of same column followed by different letters are significantly different ( $p < 0.05$ ).

**Table 8.** Effect of different break rolls (B1) gap settings on the particle size distribution (%) of semolina

Sieve (mesh size)	Break rolls gap (mm)							
	0.30	0.35	0.40	0.45	0.50	0.70	0.80	
30 (600 $\mu$ )	15.92±0.40 <sup>d</sup>	17.81±0.32 <sup>c</sup>	23.85±0.20 <sup>a</sup>	22.56±0.41 <sup>b</sup>	15.55±0.17 <sup>d</sup>	12.10±0.38 <sup>e</sup>	9.60±0.25 <sup>f</sup>	9
40 (425 $\mu$ )	20.70±0.18 <sup>g</sup>	24.67±0.35 <sup>f</sup>	28.25±0.24 <sup>c</sup>	27.15±0.11 <sup>d</sup>	32.90±0.30 <sup>a</sup>	30.25±0.32 <sup>b</sup>	26.82±0.30 <sup>d</sup>	26
50 (250 $\mu$ )	13.68±0.20 <sup>bc</sup>	12.55±0.15 <sup>d</sup>	11.12±0.36 <sup>e</sup>	11.33±0.35 <sup>e</sup>	13.35±0.38 <sup>c</sup>	14.04±0.20 <sup>b</sup>	14.00±0.28 <sup>b</sup>	14
60 (180 $\mu$ )	40.25±0.37 <sup>a</sup>	36.51±0.28 <sup>d</sup>	31.50±0.40 <sup>g</sup>	32.42±0.19 <sup>f</sup>	30.00±0.25 <sup>h</sup>	35.65±0.20 <sup>e</sup>	38.92±0.33 <sup>c</sup>	36
80 (150 $\mu$ )	5.80±0.17 <sup>a</sup>	5.05±0.21 <sup>b</sup>	4.12±0.30 <sup>c</sup>	3.85±0.15 <sup>cd</sup>	3.61±0.27 <sup>d</sup>	3.90±0.14 <sup>cd</sup>	5.00±0.25 <sup>b</sup>	5
through 100	3.16±0.23 <sup>c</sup>	2.00±0.14 <sup>e</sup>	1.27±0.20 <sup>f</sup>	2.50±0.27 <sup>d</sup>	4.11±0.31 <sup>b</sup>	3.23±0.19 <sup>c</sup>	4.82±0.22 <sup>a</sup>	5

Data are expressed as mean  $\pm$  SD. Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

semolina particles, increased. The roll gap adjustments of 0.3 mm and 0.35 mm produced semolina with a very fine particle size (Table 8) which was contaminated with very fine bran particles. Removal of bran particles from these two samples was quite cumbersome. On the other hand, 0.4 mm gap between the break rolls produced more acceptable semolina and relatively less flour than that produced with roll gap of 0.45 mm.

Semolina milling using gaps varying from 0.5 to 0.85 mm between the break rolls, produced semolina with low yield and very fine particle size. Besides, a considerable amount of large semolina particles was diverted to bran fraction that was later required to be recovered from the bran. This recovery process was not only difficult but also in some of the semolina particles the bran portion was not completely detached. Based on the above studies it was found that a gap of 0.4 mm between the pair of first break rolls (B1) and 0.2 mm between the pair of third break rolls (B3) was found to give optimum results with a reasonably high semolina yield. The resultant semolina was further purified to remove contaminated bran particles.

#### **4.1B.3. Effect of different conditioning times on semolina milling properties**

Conditioning of durum wheat for semolina milling has slightly different objective than that of flour milling. Whereas the objective in flour milling is to soften the endosperm and toughen the outer pericarp, the objective in durum milling is to toughen the bran and keep the endosperm vitreous (Posner and Hibbs, 1997). It is important that the endosperm does not get mellow and

mealy. In the present work, semolina milling was carried out to study the effects of different conditioning times, which ranged from 4 to 24 h, on semolina yield and particle size, under a constant tempering moisture of 16.5%.

Results of this investigation are shown in Tables 9 and 10. Even though, conditioning of wheat for 4 and 8 h resulted in increased semolina yield, semolina was of very fine particle size and was contaminated with lot of powdery bran particles that could not be removed during purification process. Earlier Dexter and Matsuo (1978a) showed that semolina granulation becomes finer as extraction rate increases. Since durum wheat is much harder than common wheat, it seems that 4-8 h tempering time was not sufficient to obtain optimum results. Hard wheat must be tempered longer than soft wheat since it takes more time for the water to penetrate into the hard kernel (Bizzarri and Morelli, 1988). Therefore, too short a temper time will cause the bran to shatter which will consequently affect the semolina and ultimately the pasta quality (Robinson, 2001). Increasing the conditioning time to 16 h slightly improved the semolina milling properties. However, the resultant semolina was still contaminated with excess bran particles. Though semolina from wheat tempered for 24 h was acceptable and more or less comparable with semolina from 18 h conditioned wheat, semolina yield was significantly ( $P < 0.05$ ) lower than the latter. Semolina from 24 h conditioned wheat was also slightly finer than that of 18 h conditioned wheat. Robinson (2001) pointed out that too long a temper time produces excessively fine semolina. Under the present experimental conditions, a time of 18 h was found to be more effective in producing semolina with desirable particle size

**Table 9.** Effect of different conditioning times on semolina yield\*

Conditioning time (h)	Milling fractions (%)		
	Semolina	Flour	Bran
4	82.3 ±0.44 <sup>a</sup>	6.6 ±0.22 <sup>a</sup>	11.0 ±0.31 <sup>e</sup>
8	76.6 ±0.27 <sup>b</sup>	6.1 ±0.11 <sup>b</sup>	16.8 ±0.27 <sup>d</sup>
16	74.8 ±0.29 <sup>c</sup>	5.5 ±0.33 <sup>c</sup>	20.1 ±0.32 <sup>c</sup>
18	74.2 ±0.15 <sup>c</sup>	4.2 ±0.25 <sup>d</sup>	21.5 ±0.11 <sup>b</sup>
24	72.6 ±0.41 <sup>d</sup>	3.9 ±0.26 <sup>d</sup>	23.0 ±0.40 <sup>a</sup>

\* Conditioning moisture of 16.5% and gap adjustment of rolls B1=0.4, B3=0.2mm.  
Data are expressed as mean ± SD. Means of same column followed by different letters are significantly different ( $p < 0.05$ ).



**Table 10.** Effect of different conditioning times on the particle size distribution (%) of semolina \*

Sieve No.(mesh size)	Conditioning time (h)				
	4	8	16	18	24
<b>30 (600 <math>\mu</math>)</b>	8.58 $\pm$ 0.31 <sup>e</sup>	12.51 $\pm$ 0.22 <sup>d</sup>	20.12 $\pm$ 0.36 <sup>c</sup>	23.85 $\pm$ 0.20 <sup>a</sup>	23.00 $\pm$ 0.40 <sup>b</sup>
<b>40 (425 <math>\mu</math>)</b>	21.53 $\pm$ 0.25 <sup>e</sup>	26.30 $\pm$ 0.30 <sup>c</sup>	23.01 $\pm$ 0.26 <sup>d</sup>	28.25 $\pm$ 0.24 <sup>a</sup>	27.34 $\pm$ 0.24 <sup>b</sup>
<b>60 (250 <math>\mu</math>)</b>	15.30 $\pm$ 0.21 <sup>a</sup>	13.51 $\pm$ 0.32 <sup>b</sup>	12.48 $\pm$ 0.19 <sup>c</sup>	11.12 $\pm$ 0.36 <sup>d</sup>	11.28 $\pm$ 0.33 <sup>d</sup>
<b>80 (180 <math>\mu</math>)</b>	43.32 $\pm$ 0.40 <sup>a</sup>	38.69 $\pm$ 0.35 <sup>b</sup>	34.20 $\pm$ 0.24 <sup>c</sup>	31.50 $\pm$ 0.40 <sup>d</sup>	31.05 $\pm$ 0.38 <sup>d</sup>
<b>100 (150 <math>\mu</math>)</b>	5.81 $\pm$ 0.27 <sup>a</sup>	4.54 $\pm$ 0.29 <sup>b</sup>	5.44 $\pm$ 0.24 <sup>a</sup>	4.12 $\pm$ 0.30 <sup>b</sup>	4.62 $\pm$ 0.23 <sup>b</sup>
<b>Through 100 (-150 <math>\mu</math>)</b>	5.02 $\pm$ 0.31 <sup>a</sup>	4.21 $\pm$ 0.20 <sup>b</sup>	3.71 $\pm$ 0.21 <sup>c</sup>	1.27 $\pm$ 0.20 <sup>e</sup>	2.35 $\pm$ 0.20 <sup>d</sup>

\* Conditioning moisture of 16.5% and gap adjustment of rolls B1=0.4, B3=0.2mm.  
Data are expressed as mean  $\pm$  SD. Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

required for spaghetti preparation. The semolina obtained was further purified to remove bran and other foreign particles.

#### **4.1B.4. Effect of different conditioning moisture on semolina milling properties**

Durum wheat was tempered for 18 h with moisture levels of 16, 16.5, 17, and 18%, respectively. Milling was carried out as per the standardized conditions described before. The results showed that each increase in conditioning moisture significantly reduced both the semolina and flour yield, but increased the amount of bran (Table 11). This shows that efficient separation of bran and endosperm took place by increasing the level of moisture to 18%. On the other hand, sieve analyses of semolina revealed significant ( $P < 0.05$ ) differences in semolina particle size distributions among samples (Table 12). The sieve fractions that did not vary significantly was the material held on # 60 sieve and the pan. As seen in Table 12, higher amount of moisture used for wheat tempering resulted in a significant increase in the yield of coarse semolina (held on #30 and #40 sieves), which ranged from 48.1% (16% conditioning moisture) to 57.31% (18% conditioning moisture). Based on the above results, it was found that using 17% moisture for conditioning was more suitable for milling in terms of high semolina yield, low flour yield, and effective separation of bran during milling.

**Table 11.** Effect of different conditioning moisture on semolina yield \*

Conditioning moisture (%)	Milling fractions (%)		
	Semolina	Flour	Bran
<b>16</b>	74.6 ±0.45 <sup>a</sup>	4.8 ±0.24 <sup>a</sup>	20.4 ±0.32 <sup>d</sup>
<b>16.5</b>	74.2 ±0.15 <sup>ab</sup>	4.2 ±0.25 <sup>b</sup>	21.5 ±0.11 <sup>c</sup>
<b>17</b>	73.5 ±0.36 <sup>b</sup>	3.6 ±0.25 <sup>c</sup>	23.0 ±0.34 <sup>b</sup>
<b>18</b>	72.1 ±0.41 <sup>c</sup>	3.3 ±0.24 <sup>c</sup>	24.7 ±0.21 <sup>a</sup>

\*Conditioning time of 18 h and gap adjustment of rolls B1=0.4, B3=0.2mm.  
Data are expressed as mean ± SD. Means of same column followed by  
different letters are significantly different ( $p < 0.05$ ).

**Table 12.** Effect of different conditioning moisture on particle size distribution (%) of semolina \*

Sieve No.(mesh size)	Conditioning moisture (%)			
	16	16.5	17	18
30 (600 $\mu$ )	21.50 $\pm$ 0.31 <sup>d</sup>	23.85 $\pm$ 0.20 <sup>c</sup>	28.72 $\pm$ 0.42 <sup>a</sup>	26.88 $\pm$ 0.38 <sup>b</sup>
40 (425 $\mu$ )	26.63 $\pm$ 0.30 <sup>c</sup>	28.25 $\pm$ 0.24 <sup>b</sup>	25.70 $\pm$ 0.18 <sup>d</sup>	30.43 $\pm$ 0.17 <sup>a</sup>
60 (250 $\mu$ )	11.82 $\pm$ 0.27 <sup>a</sup>	11.12 $\pm$ 0.36 <sup>a</sup>	11.53 $\pm$ 0.33 <sup>a</sup>	11.31 $\pm$ 0.26 <sup>a</sup>
80 (180 $\mu$ )	32.28 $\pm$ 0.34 <sup>a</sup>	31.50 $\pm$ 0.40 <sup>b</sup>	28.48 $\pm$ 0.32 <sup>c</sup>	26.22 $\pm$ 0.44 <sup>d</sup>
100 (150 $\mu$ )	4.51 $\pm$ 0.25 <sup>a</sup>	4.12 $\pm$ 0.30 <sup>ab</sup>	3.81 $\pm$ 0.28 <sup>bc</sup>	3.45 $\pm$ 0.09 <sup>c</sup>
Through 100 (-150 $\mu$ )	2.93 $\pm$ 0.27 <sup>a</sup>	1.27 $\pm$ 0.20 <sup>b</sup>	1.02 $\pm$ 0.20 <sup>b</sup>	0.93 $\pm$ 0.24 <sup>b</sup>

\*Conditioning time of 18 h and gap adjustment of rolls B1=0.4, B3=0.2mm.  
Data are expressed as mean  $\pm$  SD. Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

**4.1B.5. Conclusions**

On the basis of this study, the best conditions for experimental durum wheat semolina milling with a Buhler laboratory mill were found to be gap spaces of 0.4 and 0.2 mm for break rolls B1 and B3, respectively, with a tempering moisture and duration of 17% for 18 h, respectively. The above milling conditions were followed for milling of all the Indian durum varieties studied in this research work.

**Table 8.** Effect of different gap settings of break rolls (B1) on the particle size distribution (%) of semolina

Sieve No.(mesh size)	Break rolls gap (mm)							
	0.30	0.35	0.40	0.45	0.50	0.70	0.80	0.85
<b>30 (600 <math>\mu</math>)</b>	15.92±0.40 <sup>d</sup>	17.81±0.32 <sup>c</sup>	23.85±0.20 <sup>a</sup>	22.56±0.41 <sup>b</sup>	15.55±0.17 <sup>d</sup>	12.10±0.38 <sup>e</sup>	9.60±0.25 <sup>f</sup>	9.22±0.25 <sup>f</sup>
<b>40 (425 <math>\mu</math>)</b>	20.70±0.18 <sup>g</sup>	24.67±0.35 <sup>f</sup>	28.25±0.24 <sup>c</sup>	27.15±0.11 <sup>d</sup>	32.90±0.30 <sup>a</sup>	30.25±0.32 <sup>b</sup>	26.82±0.30 <sup>d</sup>	25.74±0.35 <sup>e</sup>
<b>60 (250 <math>\mu</math>)</b>	13.68±0.20 <sup>bc</sup>	12.55±0.15 <sup>d</sup>	11.12±0.36 <sup>e</sup>	11.33±0.35 <sup>e</sup>	13.35±0.38 <sup>c</sup>	14.04±0.20 <sup>b</sup>	14.00±0.28 <sup>b</sup>	14.81±0.29 <sup>a</sup>
<b>80 (180 <math>\mu</math>)</b>	40.25±0.37 <sup>a</sup>	36.51±0.28 <sup>d</sup>	31.50±0.40 <sup>g</sup>	32.42±0.19 <sup>f</sup>	30.00±0.25 <sup>h</sup>	35.65±0.20 <sup>e</sup>	38.92±0.33 <sup>c</sup>	39.50±0.24 <sup>b</sup>
<b>100 (150 <math>\mu</math>)</b>	5.80±0.17 <sup>a</sup>	5.05±0.21 <sup>b</sup>	4.12±0.30 <sup>c</sup>	3.85±0.15 <sup>cd</sup>	3.61±0.27 <sup>d</sup>	3.90±0.14 <sup>cd</sup>	5.00±0.25 <sup>b</sup>	5.31±0.15 <sup>b</sup>
<b>Through 100 (-150 <math>\mu</math>)</b>	3.16±0.23 <sup>c</sup>	2.00±0.14 <sup>e</sup>	1.27±0.20 <sup>f</sup>	2.50±0.27 <sup>d</sup>	4.11±0.31 <sup>b</sup>	3.23±0.19 <sup>c</sup>	4.82±0.22 <sup>a</sup>	5.03±0.28 <sup>a</sup>

Data are expressed as mean  $\pm$  SD. Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

#### 4.2.1. Introduction

Pasta is a traditional cereal-based food product that is becoming increasingly popular worldwide because of its convenience, nutritional quality, and palatability (Cubadda, 1994). Durum wheat (*Triticum durum*) is the best raw material for processing into pasta products due to its unique color, flavor and cooking quality (Feillet and Dexter, 1998). Pasta made from durum wheat varieties of superior quality results in a bright yellow color and it retains, after cooking, its firmness and is resistant to surface disintegration and stickiness. However, not all durum wheat semolina produces pasta of good cooking quality; many variables are involved in pasta production and their role is not completely understood (D'Egidio et al., 1990).

Many researchers have established that content and composition of proteins, gluten strength in particular, are important for the cooking quality of pasta (Matsuo and Irvine, 1970; Walsh and Gilles 1971; Matsuo et al., 1972; Grzybowski and Donnelly, 1979; Novaro et al., 1993). Wheat protein composition, i.e. protein quality, to a large extent determines the suitability of durum wheat for pasta production (MacRitchie, 1992).

Studies have also indicated that apart from gluten proteins, starch also plays an important role in determining the cooking quality of pasta. Resmini and Pagani (1983) showed that pasta cooking quality is highly influenced by both starch gelatinization and protein network formation. The role of starch in pasta cooking quality has been better understood only in recent years (Delcour et al., 2000a; 2000b; Sung and Stone, 2003).

Besides wheat components, the physical characteristics of durum wheat, such as test weight, kernel weight, kernel size, and degree of vitreousness have also been known to influence the milling performance of durum wheat and also pasta quality directly or indirectly (Dexter et al., 1987; 1988; 1991; Troccoli et al., 2000).

In the present study, six Indian durum wheat varieties, which were selected based on the initial studies discussed in Chapter 1A, were analyzed for their physicochemical, biochemical, rheological, and semolina milling properties to examine their suitability for spaghetti production.

#### **4.2.2. Physical characteristics of durum wheat varieties**

In the present study, six durum wheat varieties, namely, DWR 2006, MACS 1967, MACS 2694, PDW 215, PDW 274, and WH 896, which were selected based on their chemical characteristics reported in Chapter 1A, were examined for their physical properties. The appearance of six durum wheat kernels is shown in Fig. 33. Amongst the six varieties WH 896 had the smallest kernel size with almost opaque appearance. Other five varieties had relatively large elongated shaped kernels which is specific for durum wheat (Wiseman, 2001).

Physical characteristics of six wheat varieties are presented in Table 13. Test weight of variety PDW 274 (84 kg/hl) was significantly more than the other five varieties. This was followed by the varieties WH 896 (83.3 kg/hl) and PDW 215 (83 kg/hl). Kernels of MACS 1967 had significantly the lowest





**Fig. 33.** Kernels of the six Indian durum wheat varieties

test weight (79.75 kg/hl). These values are comparable with those of two Canadian amber durum wheat composites, which had test weights of 75.9 - 83.1 and 77.6 – 83.3 kg/hl, respectively (Dexter et al., 1987). These test weights are also much higher than those for other Indian durum varieties reported earlier by Rahim et al (1974). Dick and Matsuo (1988) reported that durum wheat having a test weight of 82 kg/hl is invariably sound and undamaged. Test weight also exhibits a strong linear relationship to kernel weight and therefore a good predictor of semolina milling (Dexter et al., 1991). Based from the above results, it can be predicted that all the above durum varieties with their relatively high test weight have the potential for good semolina yield on milling. On the other hand, Dexter et al (1987) have also pointed out that the lone beneficial effect of low test weight is an improvement in cooked spaghetti firmness and resilience, because of a strong negative relationship between test weight and wheat protein. Accordingly, it was observed that MACS 1967 that had the highest amount of protein content (15.9%) among the varieties, showed the least test weight.

Another test which is complementary to the test weight is the kernel weight. The 1000-kernel weight is a measure of average kernel size. A greater milling yield is expected with larger kernels because the ratio of endosperm to bran would be greater (Matsuo, 1988). In the present study, 1000-kernel weight varied from 40.31 g (WH 896) to 48.42 g (PDW 274). These values compare well with 24 Indian and 4 Canadian durum wheat varieties reported by Haridas Rao et al (1976). Dexter and Matsuo (1978a) reported values of 42.0 and 42.5 g for two Canadian durum wheat cultivars.

**Table 13.** Physical characteristics of durum wheat varieties

Parameter	DWR 2006	MACS1967	MACS 2694	PDW 215	PDW 274	WH 896
Test weight (kg/hl)	80.75 ± 0.29 <sup>d</sup>	79.75 ± 0.35 <sup>e</sup>	81.5 ± 0.29 <sup>c</sup>	83.0 ± 0.29 <sup>b</sup>	84.0 ± 0.5 <sup>a</sup>	83.3 ± 0.29 <sup>b</sup>
1000-kernel wt. (g)	46.72 ± 0.19 <sup>b</sup>	43.98 ± 0.56 <sup>c</sup>	41.93 ± 0.78 <sup>d</sup>	47.43 ± 0.52 <sup>b</sup>	48.42 ± 0.75 <sup>a</sup>	40.31 ± 0.70 <sup>e</sup>
Length/Breath(mm)	7.70/3.00	7.40/2.92	7.12/2.98	7.10/3.03	6.89/3.1	6.85/3.00
Vitreousness (%)	99.3 ± 0.58 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	86.6 ± 1.53 <sup>c</sup>	94.6 ± 0.58 <sup>b</sup>	92.5 ± 2.08 <sup>b</sup>	87.5 ± 2.52 <sup>c</sup>
Hardness (N)	146 ± 8.6 <sup>b</sup>	173.5 ± 9.3 <sup>a</sup>	124.8 ± 7.5 <sup>d</sup>	143.5 ± 5.8 <sup>bc</sup>	138.2 ± 8.4 <sup>c</sup>	120.0 ± 8.5 <sup>d</sup>
Falling number (s)*	583 ± 3.8 <sup>c</sup>	644 ± 2.4 <sup>b</sup>	552 ± 3.0 <sup>e</sup>	650 ± 4.1 <sup>a</sup>	577 ± 2.5 <sup>d</sup>	545 ± 1.8 <sup>f</sup>

Data are expressed as mean ± SD. Means within a row followed by different letters are significantly different ( $p < 0.05$ ).

\* 14% mb

In the present study, PDW 274 that had the highest test weight also had the highest 1000-kernel weight. On the contrary, WH 896 that had the second highest test weight showed the least 1000-kernel weight among the varieties tested. Cubadda (1988) explained that there is no valid study which demonstrates that durum wheat varieties with small kernels and hence with low 1000-kernel weight, have a potentially lower capacity to produce high semolina yields.

Measurement of the length and breadth of kernels of wheat varieties showed DWR 2006 (7.7 mm) followed by MACS 1967 (7.9 mm) to have the longest kernels. Both varieties, as was seen earlier, had lower test weight. On the other hand, varieties PDW 274 (6.89 mm) and WH 896 (6.85 mm) had relatively shorter kernels. These two varieties recorded significantly higher test weight. Matsuo and Dexter (1980b) found significant correlation between semolina yield and kernel size. However, these researchers reported that milling yield was affected only when the kernels fell below a certain size.

Kernel vitreousness is another aspect for evaluation of durum wheat quality. Non-vitreous kernels are those that are starchy, partly starchy, severely damaged, or from wheats of other classes (Matsuo, 1988). In the present study, MACS 1967 had 100% vitreous kernels followed by DWR 2006 with 99.3% vitreous kernels. Varieties MACS 2694 (86.6%) and WH 896 (87.5%) showed significantly lesser degree of vitreousness. It has been found that semolina yield reduces slightly with decrease in vitreous kernels (Dexter et al., 1988). According to Matsuo and Dexter (1980b), starchy durum wheat

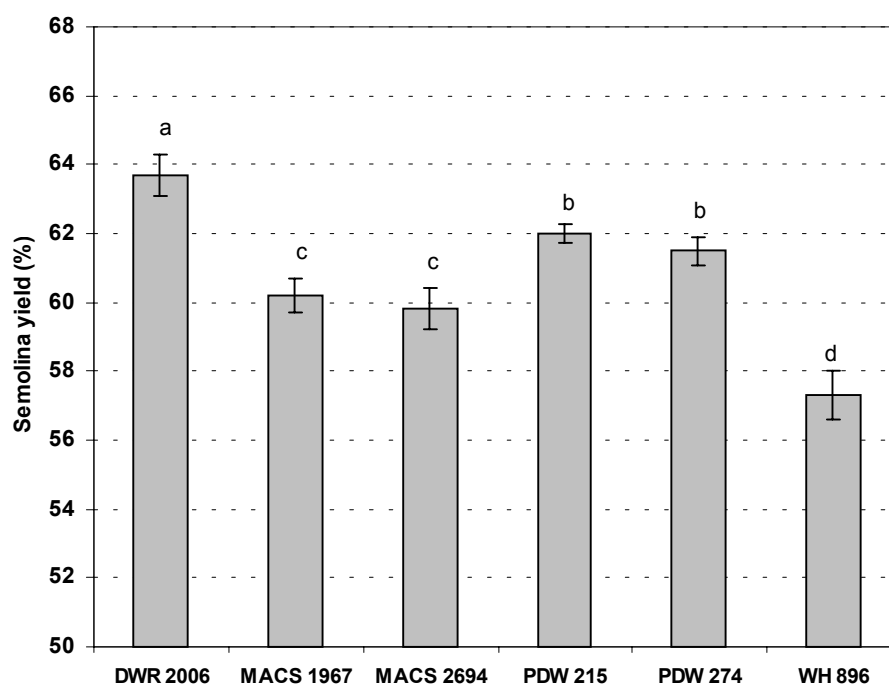
is softer than vitreous durum wheat, and gives a lower yield of semolina and a higher yield of flour, thereby reducing milling potential. Vitreous kernels are also considered to have positive effect on the color and cooking quality of pasta. However, that does not necessarily mean that semolina derived from vitreous grains always produces pasta of good cooking quality (Cubadda, 1988).

Objective measurement of the kernel hardness showed MACS 1967 to have significantly harder kernels (173.5 N) followed by DWR 2006 (146.0 N). As discussed earlier, these two varieties also had significantly higher vitreous kernels than other varieties. Hard vitreous kernels are desirable for production of semolina, whereas kernels appearing white, starchy or opaque are considered undesirable for semolina milling (Dick and Matsuo, 1988).

Falling number data for six durum varieties ranged from 545 to 650 s, which indicated that all varieties were sound without sprout damage. It has been reported that very low values for falling number (below 250 s) is the result of high levels of  $\alpha$ -amylase enzyme in semolina due to durum wheat sprout damage which can negatively influence the quality of pasta product (Donnelly, 1980; Matsuo, Dexter, and MacGregor, 1982; Turnbull, 2001b).

#### **4.2.3. Semolina milling**

Milling yield of semolina after purification and the particle size distribution of semolina are shown in Fig. 34 and Table 14, respectively. The milling yield of semolina ranged from 63.7% (DWR 2006) to 57.3% (WH 896).



**Fig. 34.** Semolina milling yield of six Indian durum wheat varieties using Buhler laboratory mill. Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

**Table 14.** Particle size distribution of semolina samples

Variety	Particle size distribution of semolina (%)					
	+500 $\mu$	+425 $\mu$	+250 $\mu$	+180 $\mu$	+150 $\mu$	-150 $\mu$
<b>DWR 2006</b>	24.5 $\pm$ 0.28 <sup>c</sup>	22.7 $\pm$ 0.20 <sup>d</sup>	11.5 $\pm$ 0.22 <sup>b</sup>	35.1 $\pm$ 0.15 <sup>b</sup>	3.2 $\pm$ 0.09 <sup>d</sup>	2.8 $\pm$ 0.10 <sup>c</sup>
<b>MACS 1967</b>	24.3 $\pm$ 0.12 <sup>c</sup>	22.8 $\pm$ 0.15 <sup>d</sup>	11.0 $\pm$ 0.20 <sup>c</sup>	35.0 $\pm$ 0.10 <sup>b</sup>	3.5 $\pm$ 0.11 <sup>c</sup>	3.1 $\pm$ 0.08 <sup>b</sup>
<b>MACS 2694</b>	25.2 $\pm$ 0.2 <sup>b</sup>	23.5 $\pm$ 0.11 <sup>b</sup>	10.3 $\pm$ 0.15 <sup>d</sup>	34.7 $\pm$ 0.25 <sup>b</sup>	3.8 $\pm$ 0.07 <sup>b</sup>	2.5 $\pm$ 0.15 <sup>de</sup>
<b>PDW 215</b>	26.0 $\pm$ 0.18 <sup>a</sup>	23.2 $\pm$ 0.21 <sup>c</sup>	12.7 $\pm$ 0.20 <sup>a</sup>	33.6 $\pm$ 0.28 <sup>c</sup>	1.5 $\pm$ 0.11 <sup>e</sup>	2.7 $\pm$ 0.07 <sup>cd</sup>
<b>PDW 274</b>	25.8 $\pm$ 0.21 <sup>a</sup>	24.3 $\pm$ 0.20 <sup>a</sup>	12.6 $\pm$ 0.09 <sup>a</sup>	32.9 $\pm$ 0.15 <sup>d</sup>	1.7 $\pm$ 0.07 <sup>e</sup>	2.4 $\pm$ 0.10 <sup>e</sup>
<b>WH 896</b>	22.4 $\pm$ 0.27 <sup>d</sup>	18.9 $\pm$ 0.15 <sup>e</sup>	10.5 $\pm$ 0.18 <sup>d</sup>	38.8 $\pm$ 0.31 <sup>a</sup>	4.1 $\pm$ 0.16 <sup>a</sup>	5.1 $\pm$ 0.12 <sup>a</sup>

Data are expressed as mean  $\pm$  SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

Durum variety WH 896 that had the lowest kernel weight, vitreousness, size and hardness, had the lowest semolina yield. Percentage of flour (-150  $\mu$ ) produced from this variety during milling was significantly higher than those of other varieties. On the other hand, variety DWR 2006 kernels, which had significantly higher kernel length, vitreousness, and kernel hardness, had a higher semolina yield (63.7%) and a lower flour content. PDW 215 and PDW 274 with significantly higher test weight and kernel weight and with similar vitreousness and hardness, showed significantly the second highest semolina yield among the varieties. Statistical analysis showed that kernel vitreousness and 1000-kernel weight were significantly correlated (1% confidence level) with semolina yield, whereas relationship between test weight and semolina yield was not significant (Table 15). There is no general consensus among researchers on the value of test weight as an indicator of wheat milling potential (Hook, 1984; Dexter et al., 1987). Dexter et al (1990), and Troccoli and Di Fonzo (1999) also did not find any correlation between test weight and semolina yield. However, Dexter and Matsuo (1978a) demonstrated that semolina extraction rate of laboratory milled durum wheats does not alter spaghetti cooking quality.

In the laboratory experimental milling, semolina yield can be as high as 60% (Matsuo, 1988). Dexter et al (1985) reported semolina yield of 66% with fairly coarse granulation. Although data for particle size distribution indicated statistically significant differences, the range in particle size among samples was small except for WH 896 which showed more of finer semolina (-250  $\mu$  and +180  $\mu$ ) than other varieties. However, it has been reported that particle



**Table 15.** Correlation coefficients between durum wheat kernel properties and semolina yield

<b>Variable</b>	<b>Correlation coeff. (r)</b>
Test weight vs. Semolina yield	-0.21 <sup>ns</sup>
1000-kernel weight vs. Semolina yield	0.81 <sup>**</sup>
Vitreousness vs. Semolina yield	0.61 <sup>**</sup>
Hardness vs. Semolina yield	0.35 <sup>ns</sup>

Statistical analysis was done on data with three determinations. \*\*: Significant at 1% confidence level; ns: Not significant.

size of semolina does not affect spaghetti quality to a great extent (Dexter and Matsuo, 1978a). Some claim that the optimum granulation range is between 140 and 500  $\mu$  (Posner and Hibbs, 1997). Further processing of semolina into pasta will be simple if particle size of semolina is uniform. If semolina contains too big particles, it will result in white specks in pasta because the short period of blending water is not sufficient for complete water penetration.

#### **4.2.4. Physicochemical characteristics of semolina**

The chemical characteristics of semolina milled from different durum varieties are shown in Table 16. The moisture content varied from 13.22 to 14.41% and the ash content from 0.79 to 0.86%. This shows that none of the semolina milled had higher ash content than the requirement of less than 1% ash in semolina according to Indian standard specification for semolina (Bureau of Indian Standards, IS-1010, 1968). Ash content is assuming an important factor in the assessment of semolina quality. A high ash content is normally associated with a longer extraction, which can produce a duller semolina color because of the presence of high-ash outer-endosperm particles. First grade semolina for pasta preparation is desired to have an ash content less than 0.9% (Cubadda, 1988). Protein content of semolina was significantly more in MACS 1967 (13.83%) followed by DWR 2006 (12.7%). Protein content of semolina is important because it influences the functional quality of pasta. Adequate amounts of gluten protein are necessary to impart to pasta the desirable attributes of mechanical strength and cooking quality (Kulkarni et al., 1987). Irvine (1971) explained that semolina samples with protein levels of 11.5-13.0% can be processed with little difficulty and

Table 16. Chemical characteristics of semolina samples

Variety	Moisture (%)	Ash (%) <sup>A</sup>	Protein (%) <sup>A</sup>	Yellow pigment (ppm)	Wet gluten (%)	AAI <sup>B</sup> protein (%)
<b>DWR 2006</b>	14.41 ±0.23 <sup>a</sup>	0.86 ±0.02 <sup>a</sup>	12.70 ±0.31 <sup>b</sup>	6.35 ±0.16 <sup>a</sup>	29.6 ±0.7 <sup>b</sup>	6.93 ±0.11 <sup>a</sup>
<b>MACS 1967</b>	13.92 ±0.15 <sup>bc</sup>	0.82 ±0.02 <sup>d</sup>	13.83 ±0.25 <sup>a</sup>	3.75 ±0.11 <sup>d</sup>	34.4 ±0.3 <sup>a</sup>	5.44 ±0.08 <sup>b</sup>
<b>MACS 2694</b>	13.22 ±0.27 <sup>d</sup>	0.83 ±0.05 <sup>c</sup>	11.81 ±0.18 <sup>c</sup>	3.12 ±0.11 <sup>e</sup>	28.8 ±0.6 <sup>b</sup>	5.30 ±0.18 <sup>b</sup>
<b>PDW 215</b>	14.10 ±0.10 <sup>ab</sup>	0.81 ±0.01 <sup>e</sup>	10.84 ±0.09 <sup>d</sup>	3.90 ±0.17 <sup>d</sup>	29.0 ±0.2 <sup>b</sup>	4.53 ±0.05 <sup>d</sup>
<b>PDW 274</b>	14.12 ±0.12 <sup>ab</sup>	0.84 ±0.04 <sup>b</sup>	11.55 ±0.12 <sup>c</sup>	5.16 ±0.20 <sup>c</sup>	25.2 ±0.8 <sup>c</sup>	4.30 ±0.14 <sup>e</sup>
<b>WH 896</b>	13.63 ±0.20 <sup>c</sup>	0.79 ±0.03 <sup>f</sup>	9.30 ±0.28 <sup>e</sup>	5.46 ±0.14 <sup>b</sup>	25.6 ±0.6 <sup>c</sup>	4.8 ±0.12 <sup>c</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

<sup>A</sup> Dry basis

<sup>B</sup> Acetic Acid Insoluble

expected to give satisfactory results. Too low a protein is likely to produce pasta with relatively poor mechanical strength in the dried product and less than optimum quality with respect to cooking stability and cooked firmness (Grzybowski and Donnelly, 1979). Among the six varieties studied PDW 215 had a significantly low protein content of 10.84% followed by WH 896 that had a protein content of 9.30%. However, percentage of wet gluten in semolina which is one of the indicators of protein quality was not in the same trend of semolina protein content. MACS 1967 that had the highest protein content also had the highest amount of wet gluten (34.4%). Though PDW 274 had considerable amount of protein (11.55%), its wet gluten content was the lowest (25.2%) among the samples. Low wet gluten content in WH 896 (25.6%) can be attributed to low amount of total protein in semolina. Though protein content of other three varieties namely, DWR 2006, MACS 2694, and PDW 215 was significantly different from each other, their wet gluten content was not different significantly. Earlier, De Stefanis and Sgrulletta (1990) found wet gluten of 22 to 34% in semolina from five Italian durum varieties whose average value (26.6%) was less than that of the six Indian durum varieties reported here (28.7%).

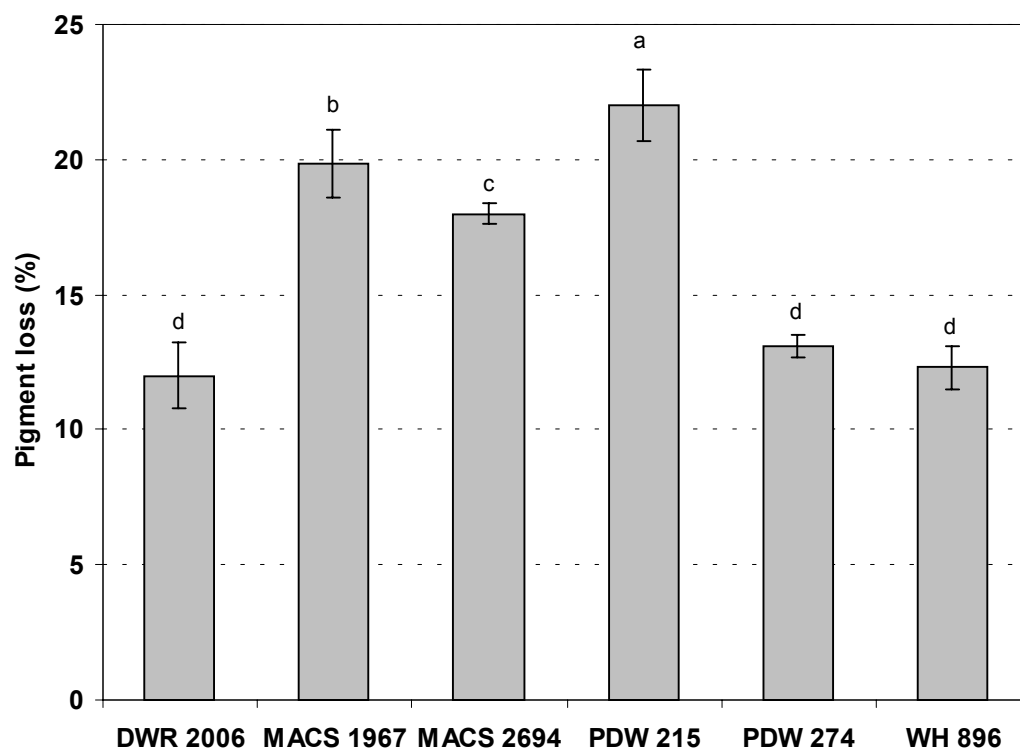
Results of the present study showed that the highest amount of acetic acid insoluble protein (6.93%) was present in the variety DWR 2006 and the lowest (4.30%) in PDW 274. Even though wheat variety MACS 2694 had the same amount of total protein as PDW 274, its acetic acid insoluble protein content was higher significantly. On the other hand, WH 896 that had the lowest protein content had significantly higher level of acetic acid insoluble

protein than few other varieties, which had higher protein content. Sgrulletta and De Stefanis (1989) found that acetic acid insoluble protein was more efficient than total protein content for predicting pasta cooking quality.

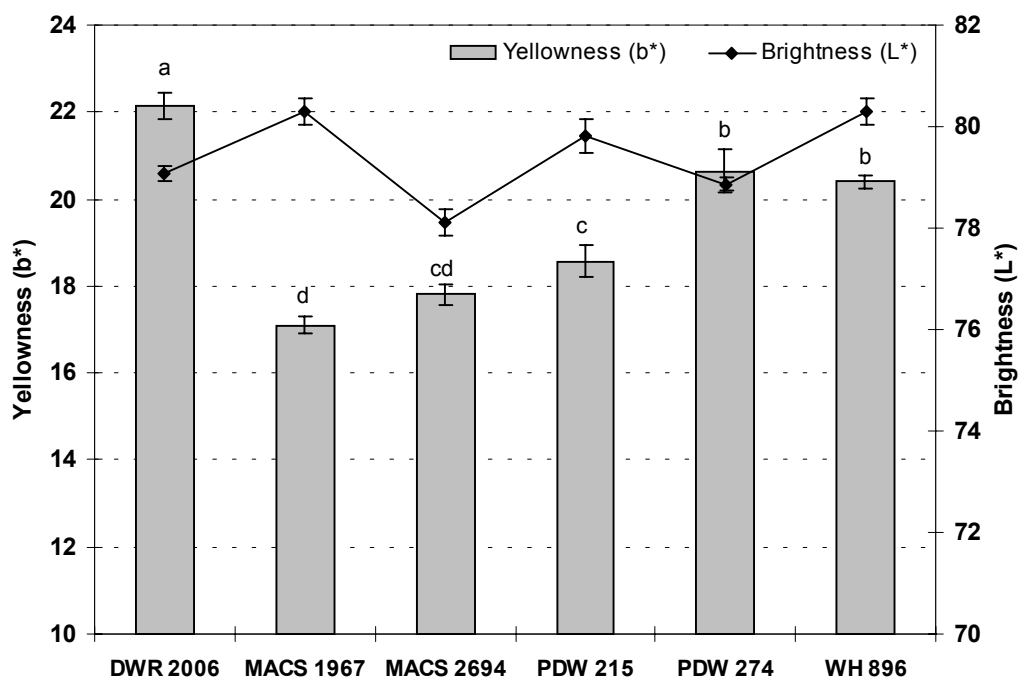
Content of yellow pigment in semolina is another important parameter determining the pasta making potential of semolina samples. This is because the color of semolina is an indication of the color one might expect in the pasta. In the present study the yellow pigment content was the highest in the semolina from variety DWR 2006 (6.35 ppm) and lowest for the variety MACS 2694 (3.12 ppm). Semolina from WH 896 had the second highest pigment content (5.46 ppm). Present study showed that there was a pigment loss ranging from 12% (DWR 2006) to 22% (PDW 215) during the milling process (Fig. 35). Earlier, Dexter and Matsuo (1978a) reported pigment loss of 17.7% and 20.3% for two Canadian amber durum wheat varieties.

#### **Semolina color characteristics**

Semolina color characteristics are shown in Fig. 36. There was no significant difference in brightness ( $L^*$  value) among different semolina samples. Manthey and Hareland (2001) found a relationship between semolina brightness and ash content. However, in the present study, ash content of semolina samples was in the narrow range of 0.79 – 0.86% (Table 16), though they were significantly different. Yellowness ( $b^*$  value) of semolina from DWR 2006 was significantly higher than that of other semolina samples (Fig. 36), which can be attributed to its higher yellow pigment content. However, Dexter and Matsuo (1978) indicated that yellow color of semolina is



**Fig. 35.** Yellow pigment loss during milling of durum wheat varieties. Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.



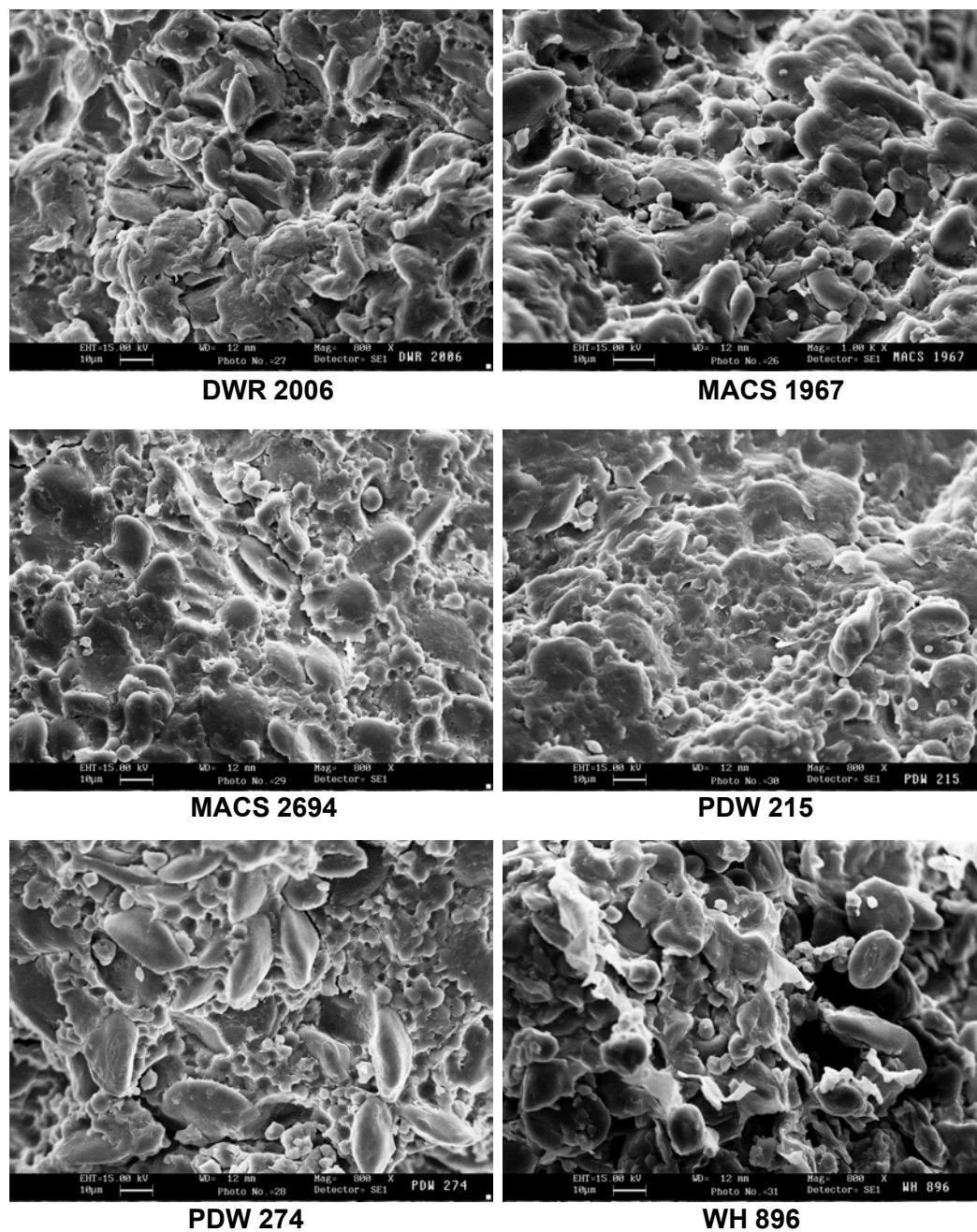
**Fig. 36.** Color characteristics of semolina samples from six durum varieties. Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

due to differences in light reflectance from fine and coarse semolina and not necessarily to yellow pigment content. As was seen earlier, the particle size distribution of semolina samples (Table 14) did not show much variation among the samples except for WH 896, which had significantly more of finer particle size. Probably due to this reason, yellowness of semolina from WH 896 was comparable to that of PDW 274, in spite of its higher pigment content.

### **Scanning electron microscopy (SEM) of semolina**

Scanning electron micrographs of semolina samples from different durum varieties are shown in Fig. 37. They appear to be composed of both large lenticular and small round starch granules embedded in an irregular shaped protein matrix. Micrographs of semolina from all varieties, except WH 896, appear to be tightly and compactly packed with no air spaces. Similar results were also observed by Dexter et al (1978). On the contrary, micrograph of the variety WH 896 showed no tight and compact structure and there seemed to be more number of exposed and damaged starch granules. The reason for the above difference has been explained as due to the hardness of the wheat kernels (Hoseney, 1992). When hard wheat is broken, it breaks at the cell wall rather than through the cell and under the SEM the strong adherence of protein to starch granules results in the appearance of tight and compact structure. On the other hand, when soft wheat is broken it fractures through the cell content rather at the cell wall hence exposing more of the embedded starch granules. SEM studies carried out by Moss et al (1980) on the soft wheat endosperm showed that the continuity of the protein





**Fig. 37.** Scanning electron micrographs of semolina from six Indian durum wheat varieties

matrix might be related to grain hardness. As was discussed earlier, the kernels of WH 896 variety had the least hardness value than the other varieties studied. This variety also had the least protein content. Micrograph of semolina from PDW 274 was also slightly different from other samples in which, majority of the large lenticular shaped starch granules with a size of around 30  $\mu$  and not covered by the protein matrix were visible. In other varieties, the maximum granule size was around 25  $\mu$ .

#### **Farinograph characteristics**

Results of farinograph experiment for semolina samples, which was carried out at 35% water absorption are shown in Fig. 38 and Table 17. The results showed that varieties MACS 1967 (3.3 min) and DWR 2006 (3.7 min) had the lowest dough development time (DDT). The maximum consistency (MC) of these two varieties was 372 and 366 FU, respectively. These two varieties had significantly higher protein content than the other varieties. On the other hand, semolina from variety WH 896 with the lowest amount of protein content showed the highest DDT (10.6 min) and the lowest MC (270 F.U.). This is supported by the work of Irvine et al (1961) which showed that as the protein content in semolina increased, farinograph DDT decreased and MC increased. Statistical analysis also showed a highly significant inverse relationship between semolina protein content and DDT. Protein content of semolina and MC were also significantly correlated. Similarly, there was significant negative correlation ( $r = -0.68^{**}$ ) between semolina wet gluten and DDT; and positive correlation ( $r = 0.69^{**}$ ) between semolina wet gluten and MC (Table 18). Though a negative correlation between acetic acid insoluble

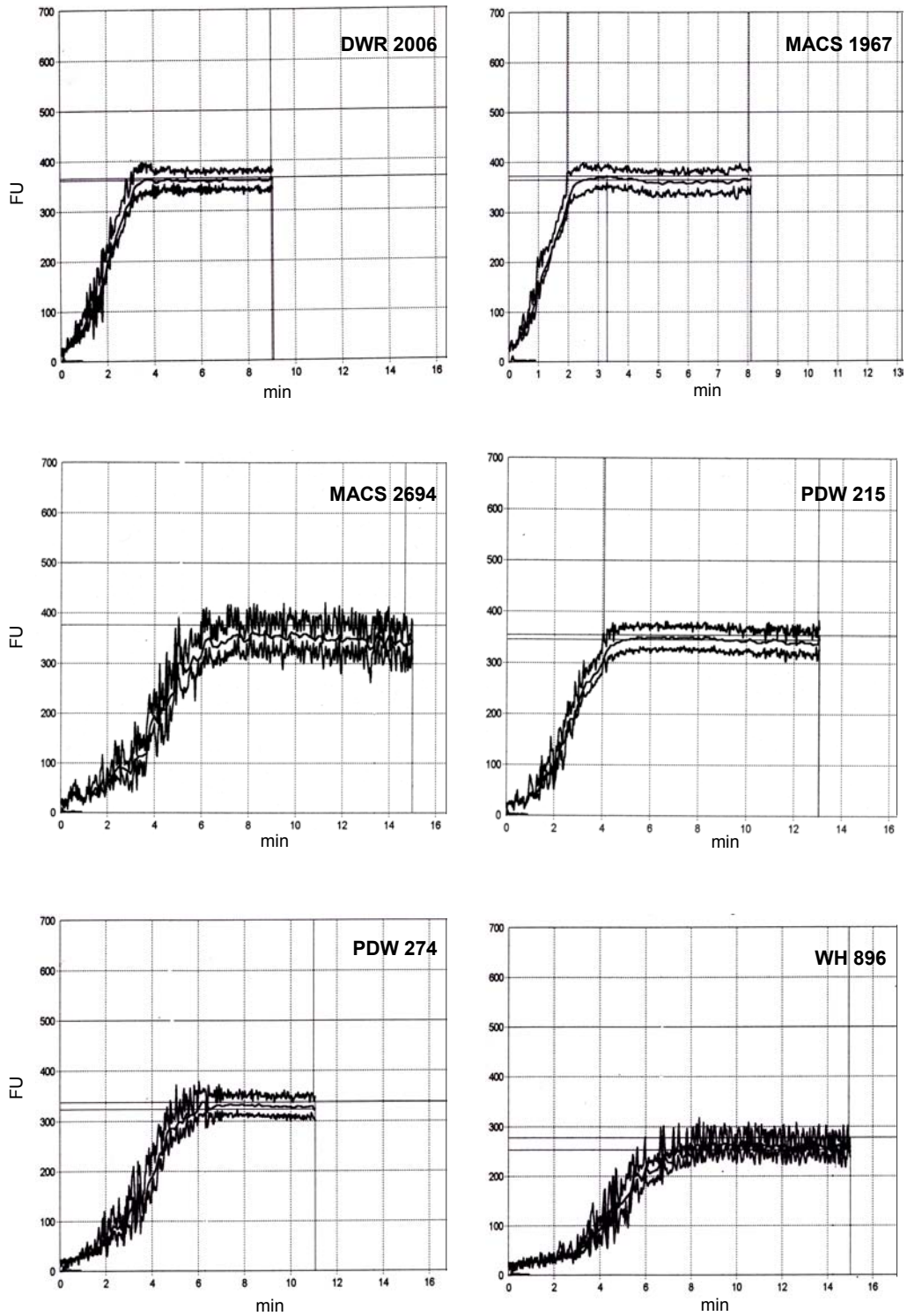


Fig. 38. Farinograms of semolina from six Indian durum wheat varieties

**Table 17.** Farinograph properties of semolina samples

Variety	Dough development time (min)	Maximum consistency (FU)	Tolerance index (FU)
DWR 2006	3.7 ± 0.22 <sup>e</sup>	366 ± 3.5 <sup>ab</sup>	6.0 ± 0.15 <sup>c</sup>
MACS 1967	3.3 ± 0.43 <sup>e</sup>	372 ± 4.6 <sup>a</sup>	15.7 ± 0.20 <sup>a</sup>
MACS 2694	8.7 ± 0.21 <sup>b</sup>	361 ± 3.1 <sup>b</sup>	6.0 ± 0.11 <sup>c</sup>
PDW 215	5.5 ± 0.35 <sup>d</sup>	349 ± 2.8 <sup>c</sup>	7.6 ± 0.12 <sup>b</sup>
PDW 274	6.3 ± 0.12 <sup>c</sup>	338 ± 3.8 <sup>d</sup>	8.0 ± 0.09 <sup>b</sup>
WH 896	10.6 ± 0.30 <sup>a</sup>	270 ± 4.0 <sup>e</sup>	15 ± 0.15 <sup>a</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

**Table 18.** Correlation coefficients between total protein, wet gluten and acetic acid insoluble protein of semolina and farinograph characteristics

Variable	Correlation coeff. (r)
Total protein vs. DDT	-0.81 **
Total protein vs. MC	0.88 **
Total protein vs. MTI	-0.05 <sup>ns</sup>
Wet gluten vs. DDT	-0.68 **
Wet gluten vs. MC	0.69 **
Wet gluten vs. MTI	0.26 <sup>ns</sup>
AAI protein vs. DDT	-0.44 <sup>ns</sup>
AAI protein vs. MC	0.44 <sup>ns</sup>
AAI protein vs. MTI	-0.21 <sup>ns</sup>

Statistical analysis was done on data with three replicates. \*\*: Significant at 1% confidence level. ns: Not significant.

protein and DDT, and also a positive correlation between acetic acid insoluble protein and MC were noticeable, they were not significant at 1% or 5% confidence level.

All semolina samples in the present study showed low values for mixing tolerance index (MTI). Irvine et al (1961) showed that utilization of semolina with increased particle size or with heterogenous particle size resulted in a decrease in maximum consistency and tolerance index. Our results agree with their findings as around 50% of semolina samples were retained on 425  $\mu$  mesh (Table 14).

#### **Pasting properties of semolina**

Pasting properties of semolina samples as measured in micro visco-amylograph are shown in Fig. 39 and Table 19. MACS 1967 had the highest onset gelatinization temperature (66.1 °C) closely followed by MACS 2694 (65.4 °C), while PDW 215 had a significantly lower onset gelatinization temperature (63.7 °C) comparable to those of PDW 274 and DWR 2006. The gelatinization temperature is related to breakdown of the hydrogen bonds between the molecules of starch and beginning of swelling of starch granules in the presence of heat and water. Differences in onset gelatinization temperature are due to the strength of bonding of the micellar network of individual starch granules. It is known from the literature (Kulp, 1973; Eliasson and Karlsson, 1983) that small wheat starch granules gelatinize at higher temperatures than the larger granules. Since wheat starch contains both large lenticular and small round granules, it could be possible that the varieties

PDW 215, PDW 274, and DWR 2006 had higher proportion of large granules that would have caused an early onset of gelatinization. On the other hand, MACS 1967 could have contained a lower proportion of large granules, hence a higher onset gelatinization temperature. However, the onset gelatinization temperature range in the present study was very narrow to be influenced significantly by the size of the starch granules.

Durum variety WH 896 showed the highest peak viscosity (912 BU) compared to other varieties. It has been reported that higher content of starch in flours, to some extent, may contribute to higher pasting viscosity (Ragaei and Abdel-Aal, 2006). Therefore, lower protein content in this variety might have resulted in higher starch concentration and hence higher peak viscosity. Higher peak viscosity may also be due to the ability of starch granules to swell more. With more swelling of the granules, the tendency is greater to leach their contents into the surrounding liquid during cooking of pasta, leading to an increase in cooking loss (Sissons and Batey, 2003). Differences in protein composition are also known to affect pasting viscosities and properties (Batey and Curtin, 2000). On the other hand, varieties DWR 2006 and MACS 1967 also had higher peak viscosities. However, these two varieties had significantly higher protein contents compared to WH 896. Breakdown values for viscosity showed varieties WH 896, MACS 2694 and MACS 1967 having significantly higher values than the other three varieties. Breakdown viscosity reflects the fragility of the swollen granules which first swell and then breakdown under the continuous stirring action of the amylograph. Therefore, these values indicate that starch from these varieties were more fragile and

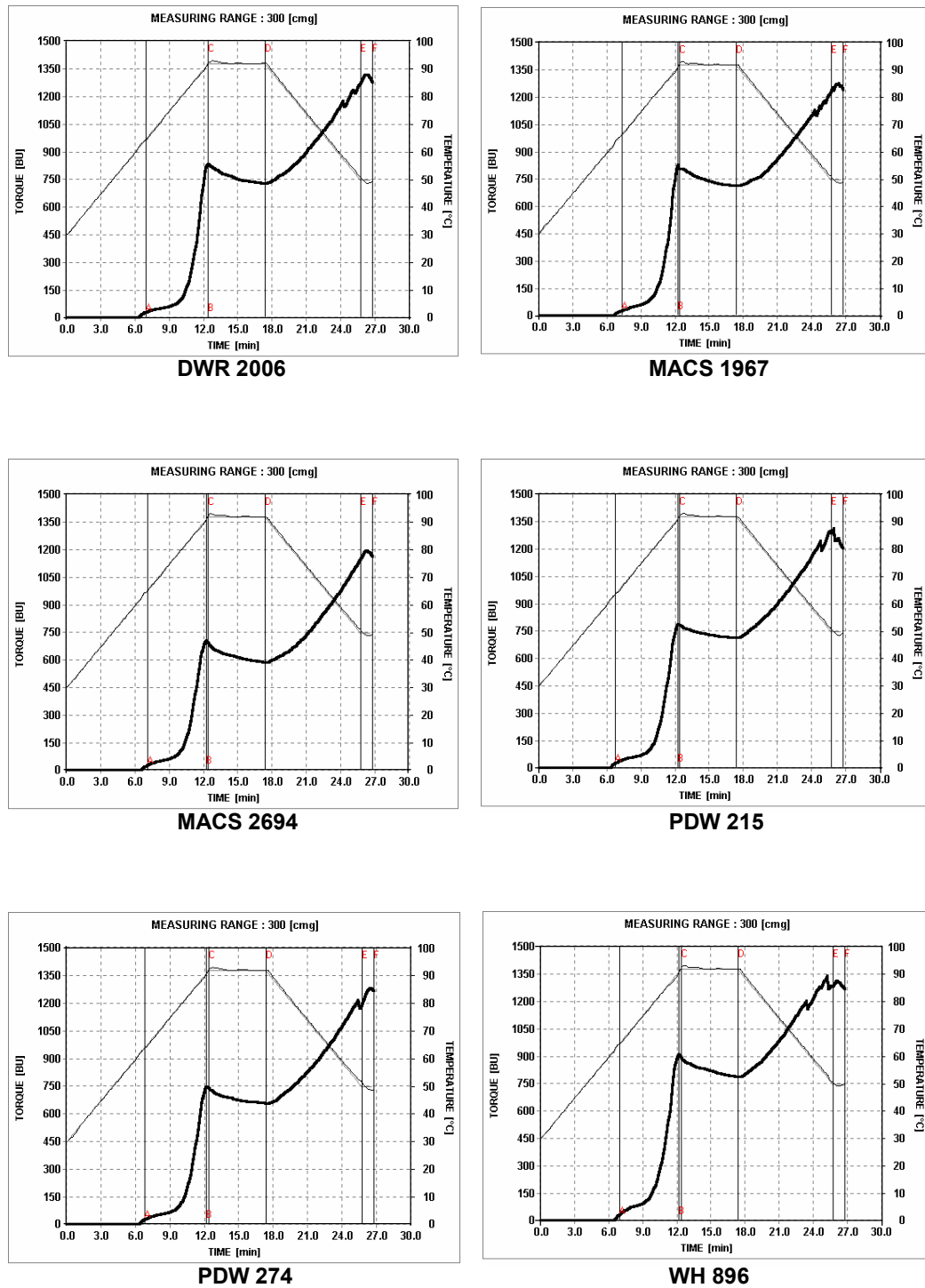


Fig. 39. Micro visco-amylograms of semolina from six Indian durum wheat varieties



Table 19. Pasting properties of semolina samples

Variety	Onset gelatinization Temp. (°C)	Peak viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)
DWR 2006	64.1 ± 0.28 <sup>cd</sup>	829.5 ± 4.9 <sup>b</sup>	98.5 ± 7.1 <sup>b</sup>	546 ± 2.8 <sup>b</sup>
MACS 1967	66.1 ± 0.07 <sup>a</sup>	828 ± 4.2 <sup>b</sup>	113.5 ± 4.9 <sup>a</sup>	520.5 ± 3.5 <sup>d</sup>
MACS 2694	65.4 ± 0.35 <sup>b</sup>	701.5 ± 4.9 <sup>e</sup>	118.5 ± 2.7 <sup>a</sup>	568.5 ± 4.9 <sup>a</sup>
PDW 215	63.7 ± 0.07 <sup>d</sup>	794.5 ± 5.0 <sup>c</sup>	72.5 ± 3.5 <sup>c</sup>	564.5 ± 6.4 <sup>a</sup>
PDW 274	64.0 ± 0.07 <sup>d</sup>	752.5 ± 4.8 <sup>d</sup>	89 ± 1.5 <sup>b</sup>	534 ± 5.2 <sup>c</sup>
WH 896	64.5 ± 0.00 <sup>c</sup>	912 ± 2.8 <sup>a</sup>	122.5 ± 2.1 <sup>a</sup>	496 ± 3.2 <sup>e</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

hence had less ability to withstand heating at high temperature and the shear stress. Ability of starch granules to withstand the shear stress applied during mixing forms an important factor in many processes (Ragae and Abdel-Aal, 2006). On the other hand, the other three varieties had significantly lower values for breakdown viscosity with variety PDW 215 recording the lowest value. Sissons and Batey (2003) pointed out that high values for breakdown are usually correlated with high peak viscosity. In the present study only MACS 1967 and WH 896, two of the three varieties with a higher peak viscosity, had a higher breakdown viscosity too, while DWR 2006 had a lower breakdown viscosity. Similarly, MACS 2694 that had a lower peak viscosity had a higher breakdown viscosity. In the present study no correlation was found between the peak and breakdown viscosities. In the present study, significant difference but not much variation was found for the set back viscosity values among the six varieties. Setback viscosity that relates to the tendency of the starch to retrograde, was significantly high in MACS 2694 (568.5 B.U.) and low in WH 896 (496 B.U.).

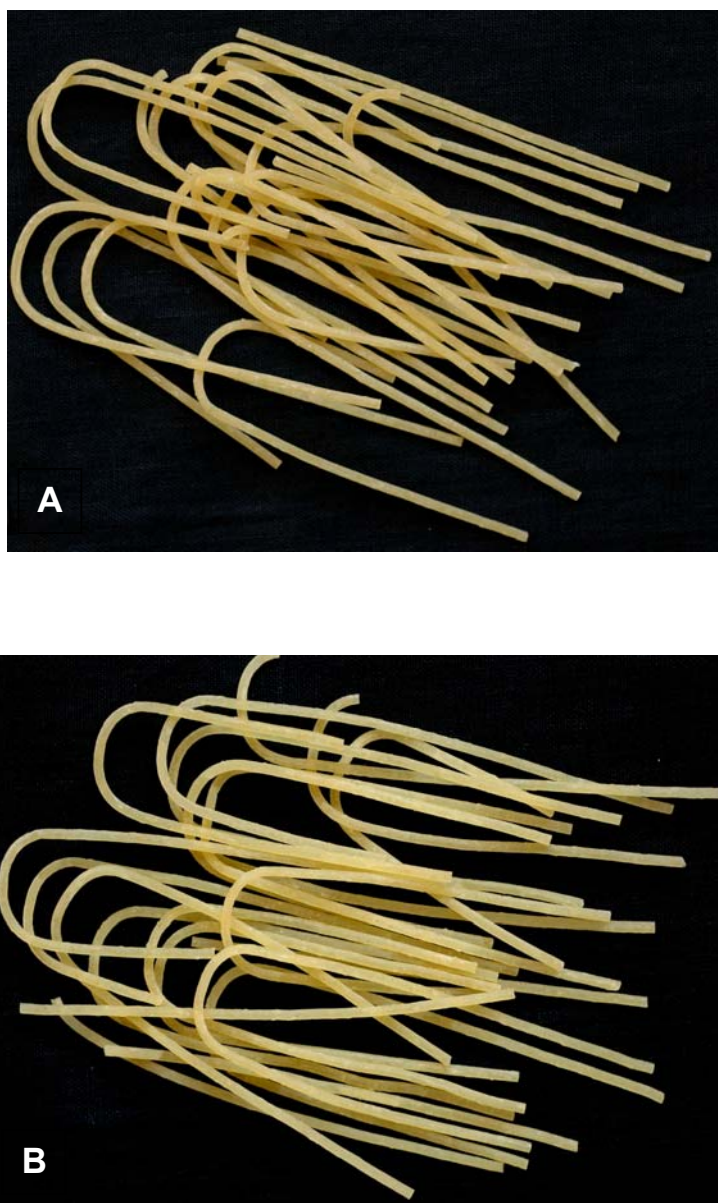
#### 4.2.5. Spaghetti making properties of Indian durum wheat varieties

Spaghetti from six durum wheat varieties was prepared following low temperature (LT) drying process to evaluate their potential for spaghetti making. It has been reported that at low drying temperatures, pasta cooking quality largely depends on the intrinsic characteristics of the raw materials (Cubadda, 1986). However, the high temperature drying technique seems to modify the semolina properties and enhance cooking quality independent of semolina quality (Resmini and Pagani, 1983; Cubadda, 1985). Therefore, for better interpretation of the results, low temperature drying process was used for the preparation of spaghetti from six durum varieties. Spaghetti samples were evaluated for their physical and cooking quality characteristics.

##### 4.2.5.1. Physical characteristics of dry spaghetti

Figure 40 shows the physical appearance of two representative spaghetti samples prepared from durum varieties DWR 2006 and PDW 274. The dried spaghetti strands from all the six varieties had a uniform yellow color and translucent appearance with a smooth and shining cross section.

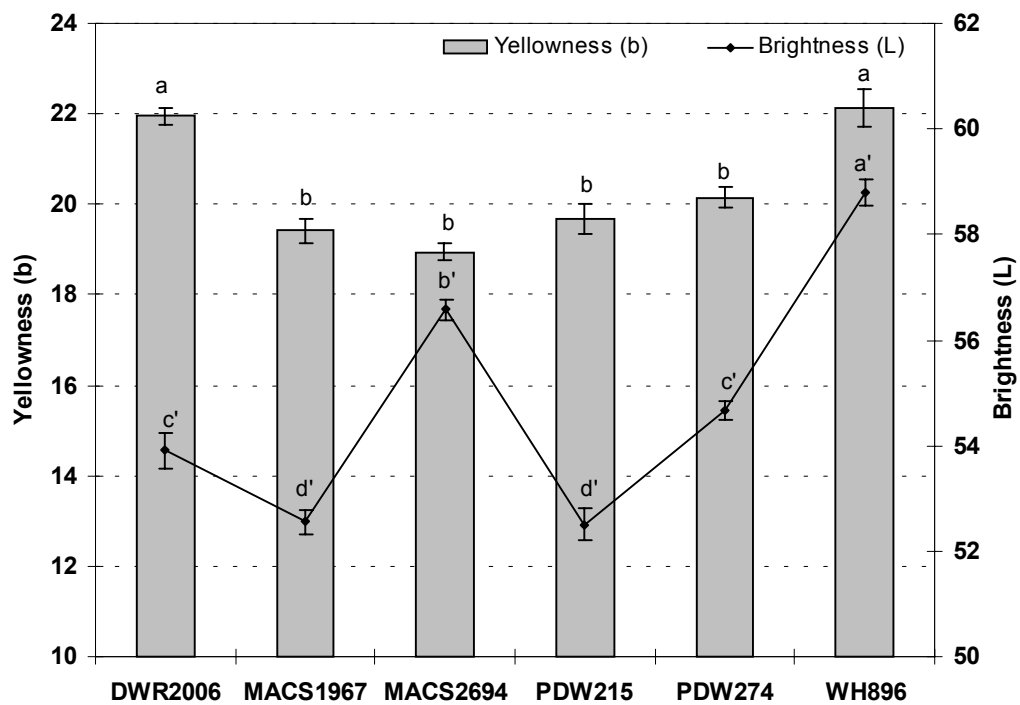
Color characteristics of dry spaghetti samples evaluated using Hunter color system, are shown in Fig. 41. Spaghetti prepared from WH 896 had the highest value for brightness (58.8) followed by MACS 2694 (56.6), while spaghetti samples from MACS 1967 and PDW 215 had the lowest values (52.5). Incidentally, semolina milled from WH 896 also had a higher brightness value. However, it should be pointed out that the color parameters measured by colorimeter are the results of surface reflectance of the sample.



**Fig. 40.** Dry spaghetti samples from durum varieties DWR 2006 (A) and PDW 274 (B) as representatives of six varieties

Therefore, it might not be appropriate to compare these characteristics between samples having different surface characteristics such as semolina in powder form and spaghetti as a solid strand having a smooth surface. Accordingly, in the present investigation, the color characteristics of semolina samples ( $L^*$  and  $b^*$ ) were measured by CIE color system and those of spaghetti samples ( $L$  and  $b$ ) by Hunter color system. Color of semolina might be affected by any of or combinations of factors such as ash content, particle size, and yellow pigment content, whereas color of spaghetti could be directly or indirectly influenced by ash content, yellow pigment content, and activity of enzymes (PPO, POD, and LOX) in semolina. One of the reasons for the brighter color of spaghetti from WH 896 could be due to significantly lower ash content in corresponding semolina.

Protein content of semolina is another factor, which might affect the brightness of spaghetti. In the present study, a significant negative correlation ( $r = -0.67^{**}$ ) was found between spaghetti brightness and total protein of semolina. This is in agreement with earlier reports (Feillet et al., 2000). Park and Baik (2004b) also found a negative relationship between brightness of noodle dough sheet and protein content of flour. The relationship between color of noodle dough and protein content of flour has also been reported in white salted noodles (Baik et al., 1995; Yun et al., 1996). The negative effect of protein content on brightness might be because of the relationship found between protein content and polyphenol oxidase activity in aestivum wheat (Baik et al., 1994; Feillet et al., 2000). Accordingly, low brightness value of spaghetti from variety MACS 1967 could be due to high polyphenol oxidase of this durum variety (Aalami et al., 2007).

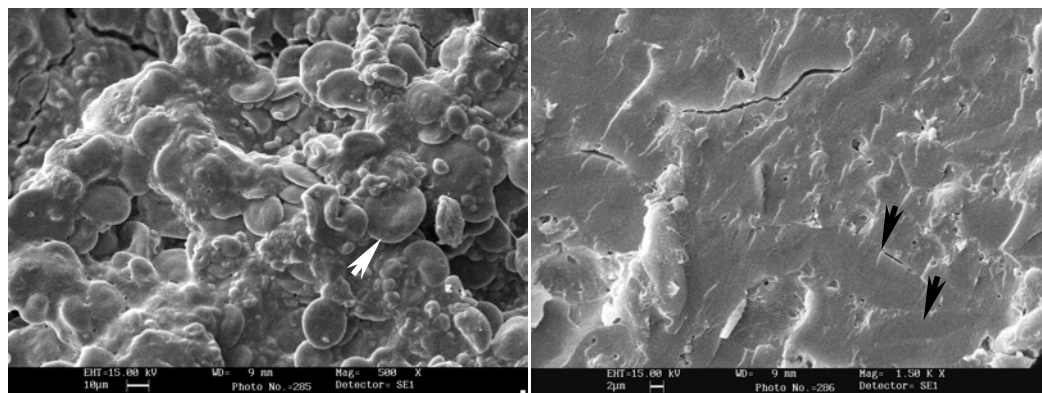


**Fig. 41.** Color characteristics of spaghetti samples from six durum varieties. Data are expressed as mean  $\pm$  SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

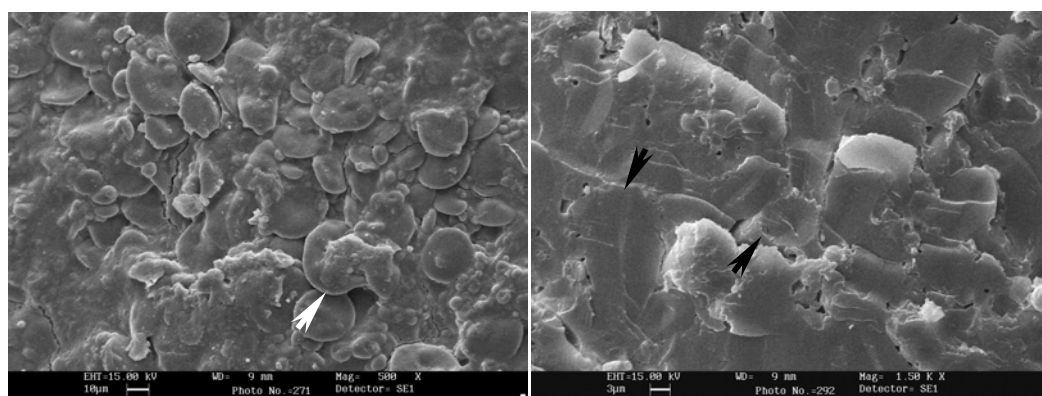
The yellow color of pasta products, rather than cooking behavior and taste, is reported to be one of the most important considerations in assessing durum wheat quality (Borrelli et al., 1999). A yellow pasta is considered a mark of quality by many consumers (Dexter et al., 1981). Yellowness (b value) of spaghetti samples are shown in Fig. 41. These values followed the trend of yellow pigment content in respective semolina samples (Table 16). Though yellowness of semolina from WH 896 was significantly lower than that of DWR 2006 (Fig. 36), yellow color of spaghetti prepared from these two varieties was not significantly different from each other. According to Turnbull (2001b), it is possible to have two semolina samples of different particle size which look different in color, but when converted to pasta is very similar in color.

#### **Scanning electron microscopy (SEM) of dry spaghetti**

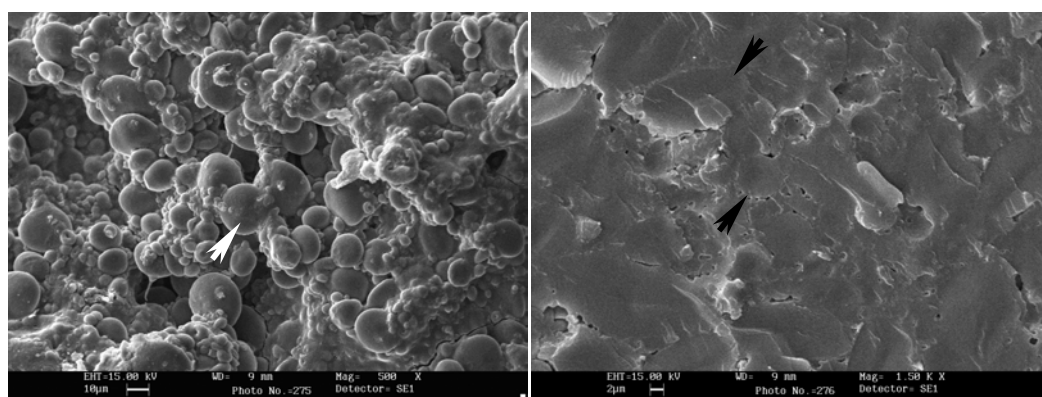
Scanning electron micrographs of surface and internal structure of spaghetti samples are shown in Fig. 42. In the outer part of dry spaghetti, numerous starch granules of varying size are visible. These starch granules appeared to be coated with a thin protein film. The extent and strength of this protein film is considered to be an important factor in spaghetti cooking quality (Dexter et al., 1978). The structure observed in the present study is similar to that which was earlier reported by Dexter et al (1978), Donnelly (1982), and Cunin et al (1995). Surface structure of spaghetti from PDW 274 and WH 896 and to some extent that of MACS 2694 appears to be different from that of the other three varieties. SEM of the outer surface of dry spaghetti from PDW 274 and WH 896 varieties showed that majority of starch granules are not covered



DWR 2006



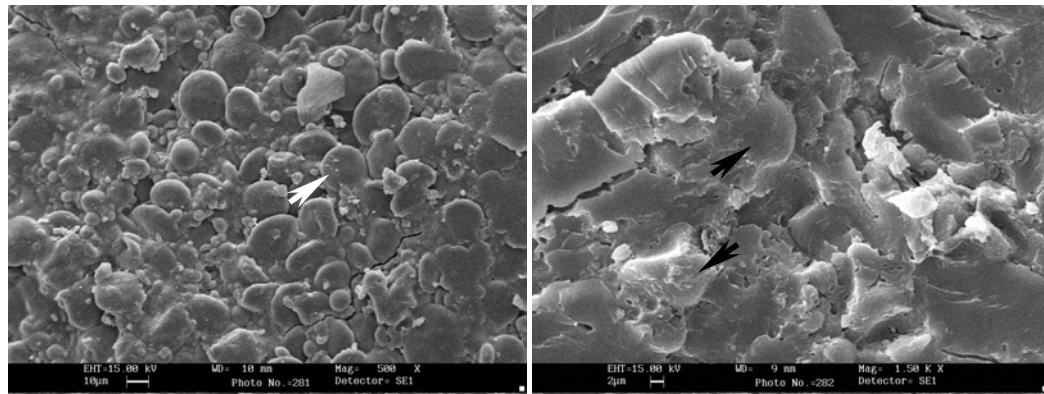
MACS 1967



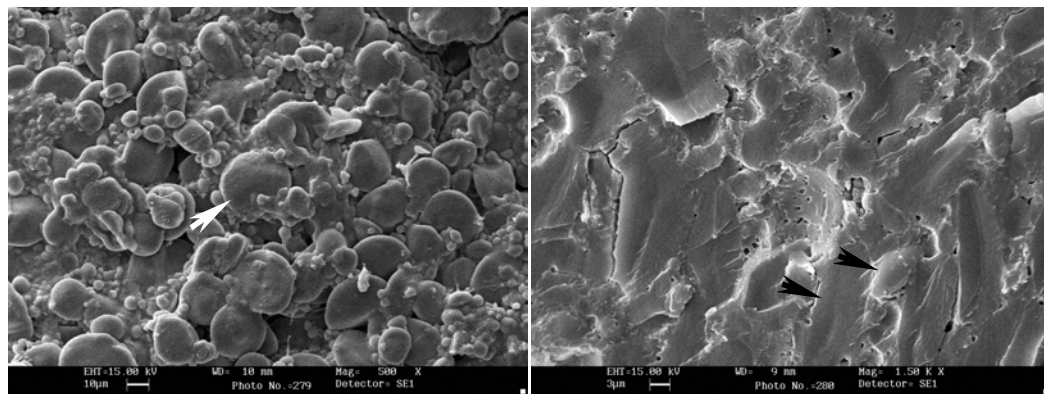
MACS 2694

**Fig. 42.** Scanning electron micrographs of surface (left) and cross section (right) of spaghetti from six Indian durum wheat varieties. Arrows show starch granules.

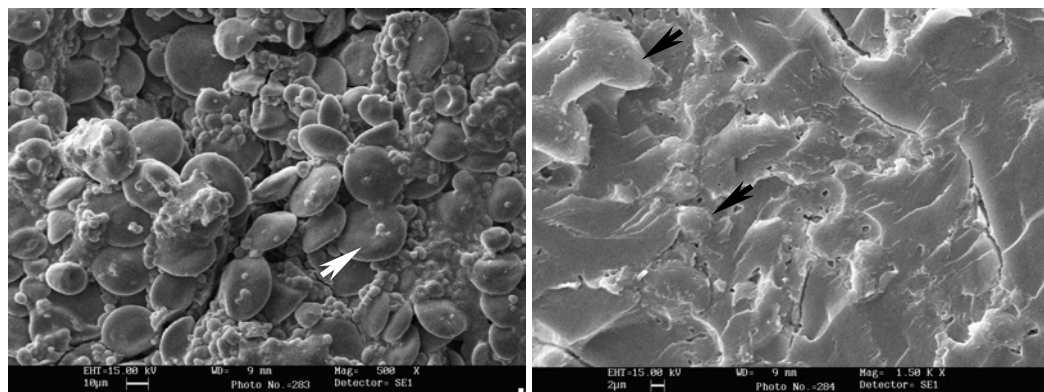




PDW 215



PDW 274



WH 896

**Fig. 42. (Continued).** Scanning electron micrographs of surface (left) and cross section (right) of spaghetti from six Indian durum wheat varieties. Arrows show starch granules.

by protein film, whereas in spaghetti from DWR 2006, MACS 1967, and PDW 215, the protein film is seen uniformly covering the surface of the samples.

Due to low moisture content in spaghetti dough and insufficient mixing during dough preparation, complete development of the gluten matrix was not observed in any of the samples. One observation which was however made was, the spaghetti samples (PDW 274, WH 896, and MACS 2694) where the starch granules were exposed rather than being embedded in the protein matrix, had relatively lower gluten content and vice versa. SEM pictures also showed many cracks and small holes in the protein matrix at the surface of all spaghetti samples. Number of these cracks appeared to be more on the surface of spaghetti from WH 896. These cracks can be partly due to shrinkage during sample preparation and partly due to tension in pasta dough during drying process (Cunin et al., 1995). Dexter et al (1978) noted that these holes and cracks can facilitate penetration of water during cooking.

Not much difference was seen in the cross-section of dry spaghetti samples. Starch granules appeared to be completely embedded in the protein matrix. The SEM showed a few intact starch granules along with some broken starch granules. It has been reported that durum and soft wheat spaghetti exhibit similar structure (Resmini and Pagani, 1983). Despite the compact structure of spaghetti cross section, many cracks and small holes were apparent in the protein matrix similar to those present in the outer part of spaghetti. According to Dexter et al (1978), these cracks and holes permit rapid penetration of cooking water into the interior part of spaghetti.

#### **4.2.5.2. Evaluation of cooking quality of spaghetti**

Table 20 shows cooking quality characteristics of spaghetti samples from the six durum varieties. All the samples were cooked for 10 min. Measurement of cooking loss of spaghetti is one of the important parameters in assessing its overall quality. Cooking loss has been associated with both starch pasting properties and protein quality (Batey and Curtin, 2000). In the present study, the lowest cooking loss was seen in spaghetti from MACS 1967 (5.8%) and PDW 215 (6.2%). One important point that needs to be emphasized here is that the above two durum varieties had entirely different physico-chemical and rheological properties. The semolina protein content of these two varieties was significantly different. While MACS 1967 had higher protein content (13.83%), PDW 215 had lower protein content (10.84%). However, it was of interest to note that the wet gluten content of PDW 215 was next only to that of MACS 1967. The amylograph characteristics of these two samples were also entirely different. While MACS 1967 had higher peak viscosity and breakdown viscosity, PDW 215 had significantly lower peak and breakdown viscosities. Statistical analysis showed a significant negative correlation ( $r = -0.79^{**}$ ) between semolina wet gluten and cooking loss of spaghetti. However, relationship between total protein and acetic acid insoluble protein of semolina with spaghetti cooking loss was not significant. It can probably be speculated that while starch properties influenced the cooking loss in PDW 215, it was either the protein content or protein quality that would have influenced the cooking loss of spaghetti made from MACS 1967. On the other hand, spaghetti made from variety PDW 274 had the highest cooking loss (8.15%). Amylograph properties showed that it had

**Table 20.** Cooking quality characteristics of spaghetti samples cooked for 10 min

Variety	Cooking loss (%)	Cooked weight (g)*	Firmness (gf)	Stickiness (N/m <sup>2</sup> )
DWR 2006	6.61 ± 0.08 <sup>c</sup>	27.8 ± 0.28 <sup>c</sup>	65.0 ± 2.0 <sup>b</sup>	554.0 ± 6.4 <sup>c</sup>
MACS 1967	5.80 ± 0.06 <sup>e</sup>	27.6 ± 0.28 <sup>c</sup>	84.0 ± 2.4 <sup>a</sup>	498.0 ± 10.8 <sup>d</sup>
MACS 2694	6.83 ± 0.10 <sup>b</sup>	27.6 ± 0.21 <sup>c</sup>	66.0 ± 1.8 <sup>b</sup>	683.2 ± 8.3 <sup>b</sup>
PDW 215	6.22 ± 0.08 <sup>d</sup>	29.6 ± 0.35 <sup>b</sup>	82.0 ± 1.9 <sup>a</sup>	550.0 ± 8.6 <sup>c</sup>
PDW 274	8.15 ± 0.11 <sup>a</sup>	30.2 ± 0.56 <sup>b</sup>	52.5 ± 2.5 <sup>c</sup>	726.5 ± 11.2 <sup>a</sup>
WH 896	6.80 ± 0.06 <sup>b</sup>	32.4 ± 0.35 <sup>a</sup>	64.5 ± 3.1 <sup>b</sup>	492.6 ± 7.6 <sup>d</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

\* From 10 g dry spaghetti

significantly lower peak and breakdown viscosities and had relatively higher protein content (11.5%), from which one could speculate a lower cooking loss. Higher cooking loss of spaghetti from PDW 274 can be partly attributed to structural properties of its semolina as observed under the SEM. Lenticular starch granules in this variety were larger than that of other varieties and were not completely covered by the protein matrix. Hill and Dronzek (1973) studying the gelatinization of wheat starch, indicated that swelling and leaching of amylose fraction was first observed in the lenticular large granules. A well developed protein matrix may prevent semolina starch from binding water and subsequent swelling. Hence, due to lack of such a protein matrix, starch has more potential to swell and breakdown the continuous gluten network during cooking, resulting in higher cooking loss (Sung and Stone, 2003).

Evaluation of spaghetti cooked weight indicated that spaghetti from WH 896 showed the highest value for cooked weight (32.4 g). Higher cooked weight of this spaghetti might be due to the higher swelling ability of starch as was discussed earlier in pasting properties of semolina. Dexter et al (1983) found a strong relationship between degree of swelling and cooked weight of spaghetti. There was not much variation among cooked weight of spaghetti from other varieties. Grzybowski and Donnelly (1979) also did not find any significant relationship between protein content and gluten strength and cooked weight.

Measurement of firmness of cooked spaghetti showed that those from two varieties, namely, MACS 1967 and PDW 215 had significantly higher

firmness values. On the other hand, spaghetti from variety PDW 274 had the least firmness. As discussed earlier, the highest amount of protein and wet gluten and the second highest amount of acetic acid insoluble protein was present in semolina from MACS 1967, whereas PDW 274 had the lowest amount of wet gluten and acetic acid insoluble protein. Firmness in cooked pasta is related to gluten strength of durum wheat (Walsh, 1971). Sung and Stone (2003) indicated that cooked pasta firmness is attributed to coagulated gluten network. Statistical analysis of present data showed a significant correlation ( $r= 0.79^{**}$ ) between wet gluten content of semolina and firmness of spaghetti samples. Dexter and Matsuo (1979b) pointed out that starch is the major component of semolina, and firmness in cooked spaghetti must, in part, be influenced by gelatinized starch properties. On the other hand, statistical analysis indicated that firmness of cooked spaghetti was negatively correlated to cooking loss ( $r= -0.91^{**}$ ) and stickiness ( $r= -0.64^{**}$ ). In other words, cooked spaghetti samples with softer texture had higher solid loss and were stickier and vice versa. Earlier, Sung and Stone (2003) found negative correlation between firmness of starch pasta and cooking loss, cooked weight and stickiness. However, in the present study there was no significant relationship between firmness of spaghetti and cooked weight.

Evaluating the stickiness properties of the cooked spaghetti, results showed that spaghetti made from MACS 1967 had significantly lower value for stickiness ( $498.0 \text{ N/m}^2$ ). On the contrary, that made from PDW 215 had significantly higher stickiness value ( $550 \text{ N/m}^2$ ). This was in spite of similarities in the cooking loss and firmness of spaghetti made from these two varieties.

On the other hand, spaghetti made from low protein variety WH 896 with higher cooking loss and lower firmness value had significantly low surface stickiness. However, the highest stickiness values were found in varieties PDW 274 and MACS 2694. Cooked spaghetti stickiness is related to the proportion of surface material that can be rinsed from the cooked spaghetti following draining (D'Egidio et al., 1982) and is not necessarily related to total solids lost to cooking water (Dexter et al., 1983). Dexter et al (1985) pointed out that high concentration of leached amylose on the surface of cooked pasta may contribute to stickiness. In the present study, a high reverse correlation ( $r = -0.84^{**}$ ) was found between semolina peak viscosity and stickiness of spaghetti. Earlier, Sissons and Batey (2003) found a negative correlation between cooking loss of pasta and amylograph peak viscosity of starch. This contradicted the hypothesis that more swelling of starch granules lead to an increase in cooking loss. Sissons and Batey (2003) suggested that factors other than starch properties, such as protein properties, could be affecting the cooking loss. Probably this explanation can be true of our results regarding negative correlation between peak viscosity and stickiness as we earlier found high correlation between spaghetti firmness and stickiness. On the other hand, a small but significant correlation ( $r = 0.49^*$ ) was observed between setback viscosity and stickiness. Earlier, Sissons and Batey (2003) claimed that they found a high correlation between stickiness and setback. Theoretically, the increase in viscosity during the cooling period of amylograph indicates the tendency of the elements present in the hot paste to associate or retrograde as the temperature of the paste decreases. The soluble amylose is largely responsible for this retrogradation phenomenon.

In this investigation, even though the variety MACS 1967, with the highest maximum consistency and lowest dough development time in farinograph exhibited excellent cooking quality characteristics, no significant correlation between farinograph data and cooking quality properties were observed. Dexter and Matsuo (1979c) have explained that true dough development might not be attained until about 45% absorption is reached in farinograph. Accordingly, Matsuo et al (1982) pointed out that mixing properties at low absorption are useful for predicting extrusion properties, but rheological properties at higher absorption where gluten is fully developed may better predict the textural characteristics of cooked spaghetti.

Pasta cooking quality can partly be predicted by studying its microstructure (Resmini and Pagani, 1983; Pagani et al., 1986). Dry pasta has a very limited water system; therefore, when cooking begins a competition starts between the starch and protein for water. If the protein surrounding the granules is less, faster will be the rate of starch swelling and gelatinization (Marshall and Wasik, 1974; Derby et al., 1975). On the other hand, if protein coagulation is more rapid than the starch swelling and gelatinization and the protein network is strong enough and uniformly distributed in the granule interspaces, the cooked pasta will be firm (Banasik et al., 1976; Dronzek et al., 1980; Moss et al., 1980). On the contrary, if starch swelling and gelatinization overcomes protein interaction, pasta will be soft and usually sticky. The larger starch granules are also another detrimental factor for pasta cooking quality because they reduce the gelatinization temperature (Resmini and Pagani, 1983; Pagani et al., 1986). Looking at the



microstructure of spaghetti samples in the present study, one can expect poor cooking quality for spaghetti from PDW 274 and WH 896, and good cooking quality for DWR 2006, MACS 1967 and PDW 215. Results of sensory evaluation and cooking quality confirmed this speculation.

Results of the cooking tolerance of spaghetti cooked for 20 min is shown in Table 21. The results showed that there was an increase in the cooking loss in all the samples on extending the cooking time. However, spaghetti from the variety PDW 274 that showed the highest cooking loss when cooked for 10 min also showed the highest cooking loss after 20 min. Similarly, MACS 1967 that had the lowest cooking loss after 10 min cooking also had the lowest cooking loss here. With the extension of cooking time cooking loss increased by 25 – 37% compared to those cooked for 10 min. Similarly, cooked weight of all these spaghetti samples increased with increase in cooking time. Sample WH 896, which had the highest cooked weight after 10 min cooking, had also the highest cooked weight after 20 min cooking. MACS 1967 and MACS 2694, which had the lowest cooked weight also showed the lowest cooked weight after 20 min cooking. Evaluation of the firmness of spaghetti strands after extended cooking showed that these values had decreased compared to those observed after 10 min cooking. The most significant change was seen in sample WH 896 whose firmness value had reduced by about 30% on overcooking. This shows that spaghetti from WH896 had the least tolerance to overcooking. This may probably be related to the low level of protein present in this variety.

**Table 21.** Cooking quality characteristics of spaghetti samples cooked for 20 min

Variety	Cooking loss (%)	Cooked weight (g)*	Firmness (gf)
DWR 2006	8.55 ± 0.07 <sup>d</sup>	36.4 ± 0.41 <sup>d</sup>	58.3 ± 1.9 <sup>b</sup>
MACS 1967	7.91 ± 0.13 <sup>f</sup>	35.9 ± 0.25 <sup>d</sup>	66.2 ± 2.5 <sup>a</sup>
MACS 2694	9.30 ± 0.11 <sup>b</sup>	36.4 ± 0.32 <sup>d</sup>	58.5 ± 2.6 <sup>b</sup>
PDW 215	8.30 ± 0.10 <sup>e</sup>	38.2 ± 0.30 <sup>c</sup>	61.5 ± 3.1 <sup>ab</sup>
PDW 274	10.21 ± 0.04 <sup>a</sup>	40.3 ± 0.25 <sup>b</sup>	44.7 ± 1.8 <sup>c</sup>
WH 896	9.10 ± 0.10 <sup>c</sup>	43.3 ± 0.28 <sup>a</sup>	45.2 ± 3.2 <sup>c</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

\* From 10 g dry spaghetti

#### 4.2.5.3. Sensory evaluation of cooked spaghetti

Cooking quality is the ultimate test of great importance, which decides the suitability of that particular durum wheat variety for spaghetti preparation. The results of the sensory evaluation of cooked spaghetti samples from the six Indian durum varieties are shown in Table 22. The spaghetti samples were evaluated for stickiness, bulkiness, firmness and the total score was based on the above three properties. Sensory score for stickiness of spaghetti, a parameter which is based on the surface disintegration of cooked sample, was the highest for spaghetti from MACS 1967, followed by DWR 2006 as well as PDW 215. High score relates to almost absence of surface stickiness, a highly desirable quality in a good quality spaghetti. As discussed previously, objective evaluation of spaghetti strands for stickiness had also shown significantly low values for samples from MACS 1967 followed by spaghetti from DWR 2006 and PDW 215. Spaghetti from variety PDW 274 showed lowest sensory score for stickiness. This was also supported by significantly high values for stickiness as measured by texture analyzer. An exception in this group was the spaghetti made from variety WH 896. Even though it had the least value for objective measurement of stickiness, denoting that the product was relatively less sticky, it had the second lowest sensory score for stickiness. On the other hand, cooked spaghetti from WH 896 was judged as a weak and less elastic sample and showed the lowest bulkiness score and second lowest firmness score. Earlier, D'Egidio et al (1993) found correlation between elasticity and stickiness. Dexter et al (1983) observed that reduced firmness and loss of resilience could conceivably mask any improvement in cooked spaghetti stickiness during taste panel assessment, though instrumental

**Table 22.** Sensory evaluation of spaghetti samples <sup>a</sup>

Variety	Stickiness	Bulkiness	Firmness	Total score <sup>b</sup>
DWR 2006	86.6	90	73.3	83.3
MACS 1967	90	93.3	90	91
MACS 2694	76.6	73.3	83.3	77.7
PDW 215	86.6	86.6	93.3	88.8
PDW 274	60	73.3	56.6	63.3
WH 896	73.3	70	66.6	70

<sup>a</sup> Data for each quality attribute is mean of five values.

<sup>b</sup> ≤ 40 = poor quality; > 40-50 = not completely satisfactory; > 50-70 = fair; > 70-80 = good; > 80 = excellent.

assessment showed less stickiness. Accordingly, it can be inferred that reduced overall textural quality of cooked spaghetti from WH 896 had negatively affected the panelist judgment for stickiness, resulting in low stickiness score for this spaghetti. The second parameter studied was the bulkiness of cooked spaghetti, which is defined as the degree of adhesion of pasta strands after cooking, more the adhesion bulkier the sample. This property depends on the discreteness of the spaghetti strands. If the cooked spaghetti strands are discrete and not sticking to each other the apparent bulkiness of spaghetti is less. On the other hand, if the spaghetti strands are not discrete but are sticking to each other the apparent bulkiness is more. Good spaghetti will have less bulkiness property and vice versa. In the present study, highest score for bulkiness was once again for spaghetti samples from MACS 1967 followed by DWR 2006 and PDW 215. Lowest value for bulkiness was scored by spaghetti from WH 896 followed by those from MACS 2694 and PDW 274. Third important property of cooked spaghetti evaluated was firmness, which is defined as the resistance of cooked pasta when chewed or flattened between the fingers or sheared between the teeth (Cubadda, 1988). Spaghetti from variety PDW 215 scored the highest for firmness closely followed by MACS 1967. Objective evaluation of spaghetti from these two varieties had also shown high values for firmness. Lowest sensory score for firmness was recorded for PDW 274. This was once again supported by objective measurement for firmness. Spaghetti from WH 896 also had a low firmness score. Panelists described this spaghetti as a breakable sample. Spaghetti from MACS 1967 had the highest total score closely followed by that from PDW 215, while spaghetti from variety PDW 274

had the lowest total score followed by WH 896. Based on the above evaluation and scores, varieties MACS 1967, PDW 215, and DWR 2006 produced spaghetti with excellent cooking quality, while varieties MACS 2694 and WH 896 produced spaghetti having good cooking quality and that from PDW 274 had fair cooking quality. None of the spaghetti samples had either 'not completely satisfactory' or 'poor' sensory quality.

As was seen from the above results spaghetti having excellent cooking quality was produced from durum wheat variety MACS 1967. The superior cooking quality of this variety can be attributed initially to the significantly high protein and gluten contents in the semolina. Similarly, variety DWR 2006 also produced good quality spaghetti, which can once again be attributed to the significantly high protein and gluten contents in the semolina. This agrees with the results of D'Egidio et al (1979) who obtained a significant positive correlation between semolina protein and pasta cooking quality. Del Nobile et al (2005) explained that an increase in protein content leads to a decrease in spaghetti stickiness mainly because it offers better resistance to overcooking. Feillet et al (1989) hypothesized that higher protein content will help in the formation of a tight protein network which will prevent starch leaching during cooking hence promotes retention of good surface condition of pasta during cooking. Low protein content in spaghetti from WH 896 might have imparted a breakable and less elastic texture to the cooked spaghetti. An exception here is the variety PDW 215, which had significantly low protein content but had high sensory score. It also had high value for objective measurement of firmness and low value for stickiness. Incidentally, this sample had

significantly high wet gluten content. Matsuo and Irvine (1970) reported that protein content of flour or semolina is not necessarily related to firmness in cooked spaghetti. The good cooking quality of pasta has also been related to starch gelatinization properties (Resmini and Pagani, 1983; Vansteelandt and Delcour, 1998; Delcour et al., 2000a,b). During cooking of pasta, the starch granules, which are present inside the gluten network, get gelatinized and occlude the interspaces. When the interaction of the coagulating protein with itself is more rapid than the starch swelling and gelatinization, the cooked pasta will be firm (Delcour et al., 2000a,b). This would result in low cooking loss. Quality evaluation of cooked pasta had shown that cooking loss was significantly very less in MACS 1967 followed by PDW 215 and DWR 2006.

Results of sensory evaluation and spaghetti cooking quality revealed that varieties MACS 1967 and PDW 274 could be considered as excellent and poor durum varieties, respectively in relation to their spaghetti making quality. Based on these results, the above two varieties namely, MACS 1967 and PDW 274 were selected for further studies, which will be discussed in the next two chapters.

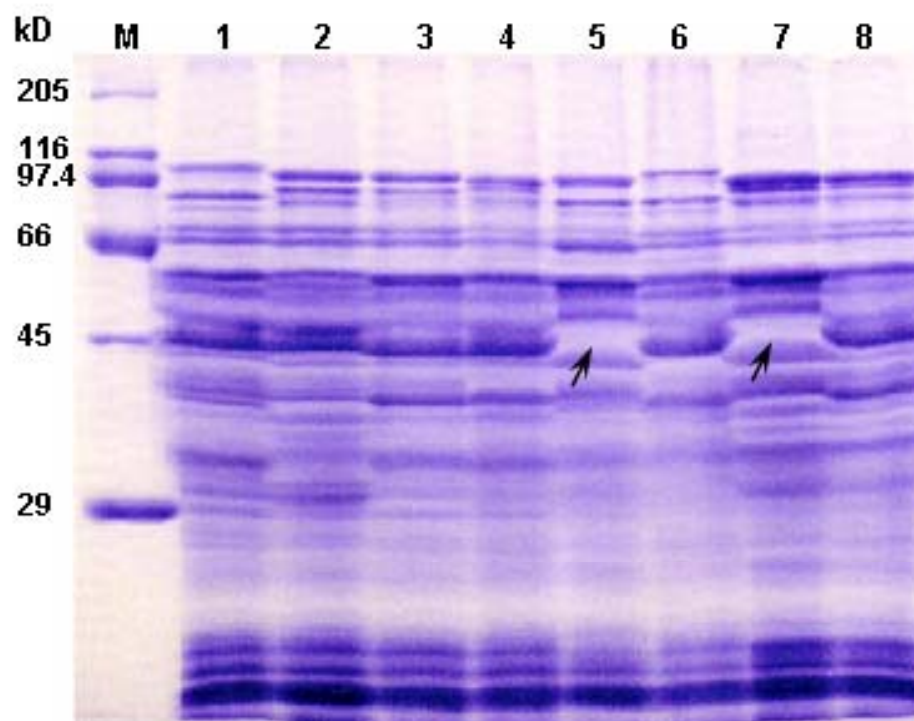
#### 4.2.6. Biochemical characteristics of durum wheat varieties

##### SDS-PAGE studies

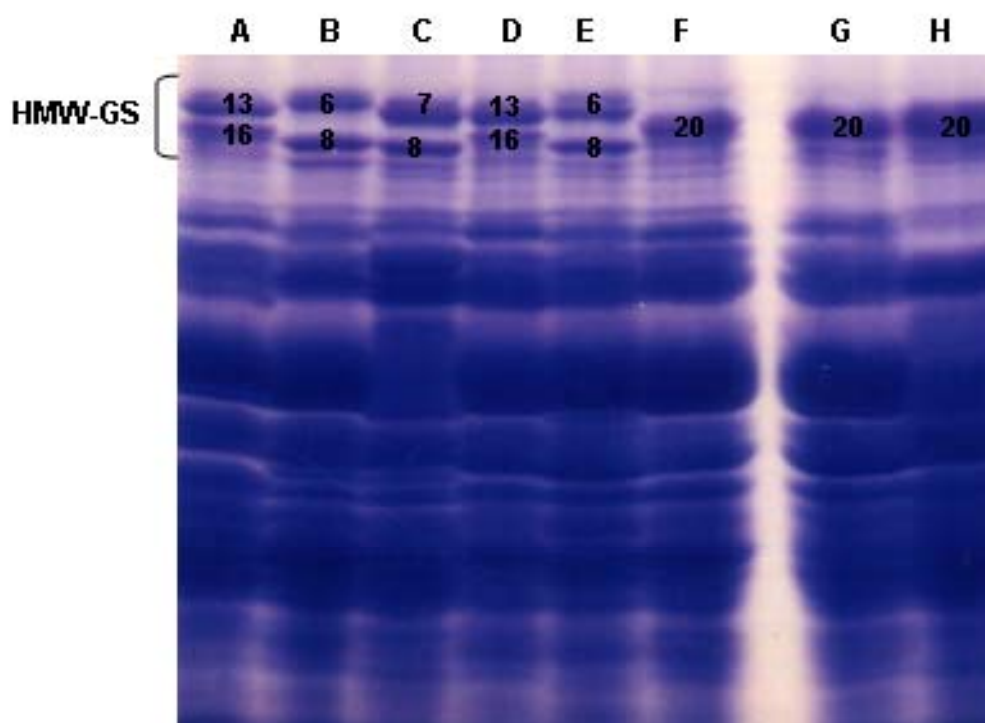
A 12% gel was used for fractionation of total flour proteins to provide better separation of subunits in the low molecular weight region (Fig. 43). Lira 42 and Lira 45, Italian durum wheats known for their poor and good spaghetti making quality, respectively (Masci et al., 1995), were used as reference wheat samples in this study. SDS-PAGE analysis of 8 varieties indicated that a protein with molecular weight around 45 kDa was absent in poor varieties PDW 274 and Lira 42, whereas proteins with molecular weight around 52 and 58 kDa appeared in higher intensity in these two varieties as compared to good varieties.

High molecular weight glutenin subunits (HMW-GS) of six Indian along with two well characterized known Italian durum wheats were identified on a 10% SDS-PAGE (Fig. 44). As reported earlier (Ch. 1A), four main subunit combinations 6+8, 13+16, 20, and 7+8 were found in six Indian durum wheats, whereas Italian variety Lira had subunit 20. There are contradictory reports regarding the relationship between glutenin subunit composition and cooking quality of pasta (Feillet, 1988). No clear relationship was found by Du Cros et al (1982) or by Autran (1981), with regard to HMW-GS and pasta quality. However, Galterio et al (1993) and Fares et al (1997) showed the positive effect of LMW- and HMW-GS on pasta quality. Autran and Feillet (1987) reported that HMW-GS 6+8 were positively associated with quality, whereas subunits 13+16 were negatively associated. In the present study, it





**Fig. 43.** SDS-PAGE fractionation of total endosperm proteins of (1) DWR 2006, (2) MACS 1967, (3) MACS 2694, (4) PDW 215, (5) PDW 274, (6) WH 896, (7) Lira42, (8) Lira45. M; Molecular weight marker. Arrows show the 45 kD polypeptide which is absent in poor durum varieties



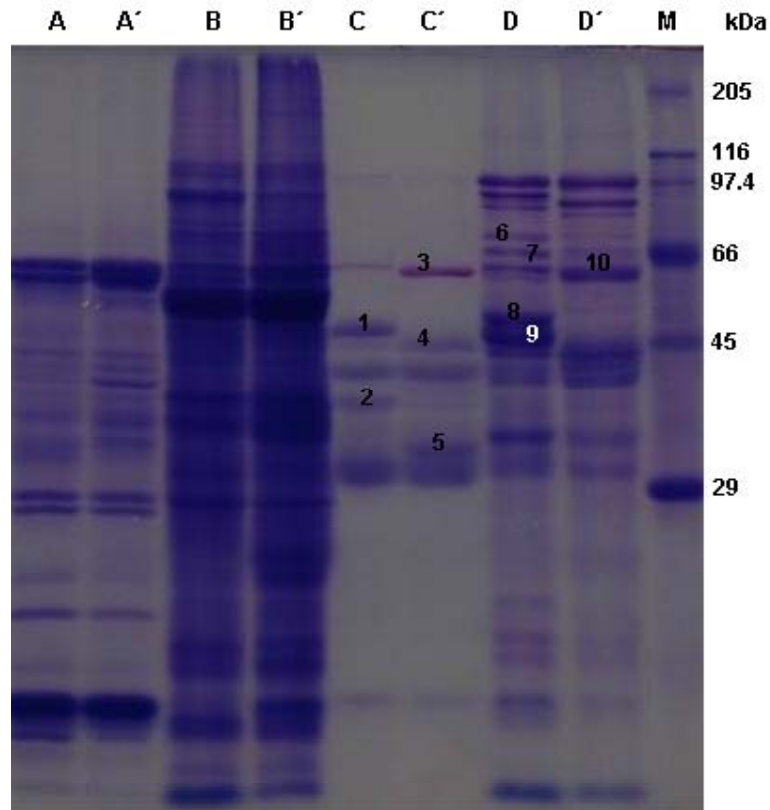
**Fig. 44.** SDS-PAGE pattern of total endosperm proteins of six Indian and two Italian durum wheats for identification of HMW-GS. (A) MACS 1967, (B) DWR 2006, (C) PDW 274, (D) MACS 2694, (E) WH 896, (F) PDW 215, (G) Lira45, (H) Lira42.

was observed that PDW 274 with HMW subunits 7+8 showed a poor cooking quality in its corresponding spaghetti, whereas spaghetti from MACS 1967

with combination of 13+16 showed the best cooking quality characteristics among the varieties studied. Therefore, according to the literature reports and the results of present investigation, it seems that HMW-GS do not have a direct effect or do not play a major role in determining the spaghetti quality.

### **SDS-PAGE analysis of protein fractions**

As it was mentioned earlier, among the six Indian durum varieties studied, MACS 1967 and PDW 274 were found to be excellent and poor, respectively, for spaghetti production. Moreover, a significant difference was noticed in the SDS-PAGE patterns of whole wheat flours of good and poor varieties. In order to find out the nature of proteins involved in this difference, wheat flours from varieties MACS 1967 and PDW 274 were fractionated into albumins, globulins, gliadins, and glutenins. The freeze-dried fractions were subjected to a 10% SDS-PAGE. The results indicated that no significant differences existed between albumin and globulin fractions of good and poor durum varieties (Fig. 45). However, a gliadin band (band #1) corresponding to MW 45 kDa was absent in poor durum variety PDW 274. A gliadin band (band #2) with MW around 39 kDa was present in MACS 1967 with significantly higher intensity than that of PDW 274. On the other hand, two gliadin bands corresponding to MW 42 kDa (band #4) and MW 33 kDa (band #5) were present in poor variety PDW 274, while they were absent in MACS 1967. Similarly, clear differences were observed between glutenin subunits of these two durum varieties. Two glutenin bands corresponding to MW 53 kDa (band



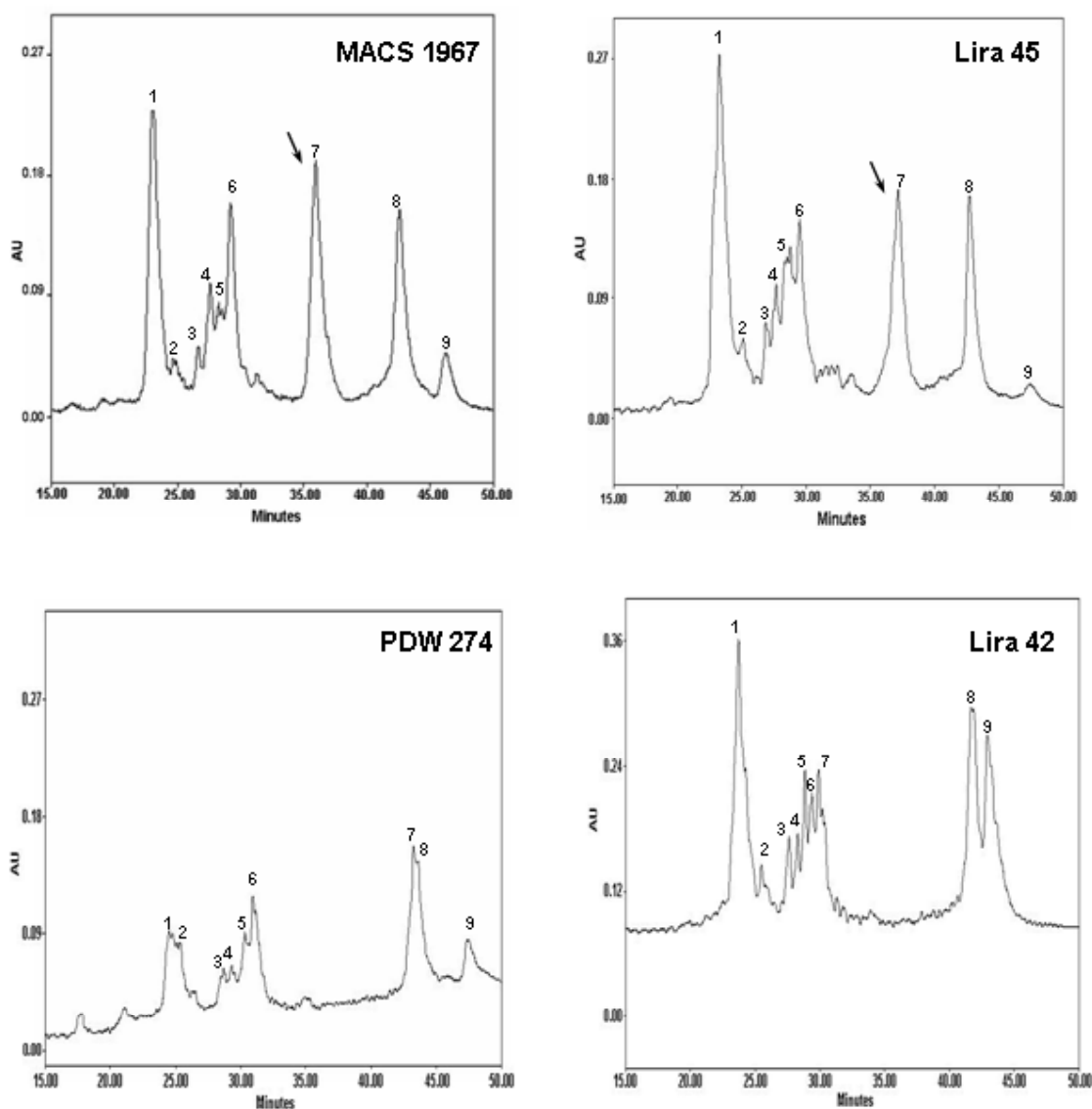
**Fig. 45.** SDS-PAGE (10% gel) patterns of four fractions of proteins from good variety MACS 1967 (**A – D**) and poor variety PDW 274 (**A' – D'**). **A & A'**: Albumins; **B & B'**: Globulins; **C & C'**: Gliadins; **D & D'**: Glutenins; **M**: Molecular weight markers.

#8) and MW 45 kDa (band #9) were absent in poor variety PDW 274. In both gliadin and glutenin fractions, protein bands with MW around 61 kDa (bands

#3 and 10) were significantly more intense in poor variety compared to good variety. Moreover, two glutenin bands (#6 and 7) corresponding to MW of around 73 and 66 kDa, respectively, were observed in MACS 1967 in significantly higher intensity than in PDW 274.

### **RP-HPLC of gliadins**

Gliadins extracted from eight different durum wheat varieties were subjected to RP-HPLC column. These proteins were clearly separated into 9-10 peaks depending on the variety (Fig. 46). Both qualitative and quantitative differences existed among the chromatograms and each possessed a unique pattern. However, a peak obtained around 36<sup>th</sup>-37<sup>th</sup> minute (designated as GliPK36-37) was found to be present in varieties with good spaghetti making quality (MACS 1967), while it was absent in the variety which yielded poor quality spaghetti (PDW 274). Similar trend was observed between the two Italian durum wheats. Lira 45 which is reported to be good for pasta making, showed the presence of GliPK36-37, while this peak was absent in Lira 42 which is reported to be poor for pasta making. The area percentage of the above mentioned peak in Indian varieties varied from 14.6% (PDW 215) to 23.2% (MACS 1967), while in Lira 45, its percentage was 26.2% (Table 23). Thus, the differences in RP-HPLC patterns of durum wheat varieties can be used as a marker to differentiate between durum varieties having good and poor pasta making properties.



**Fig. 46.** Typical RP-HPLC profiles of gliadin proteins from two Indian durum varieties, MACS 1967 (good variety) and PDW 274 (poor variety); and two Italian durum wheats, Lira 45 (good variety) and Lira 42 (poor variety). Arrows indicate the peak which is absent in poor varieties.

**Table 23.** Total peak area and retention time of the RP-HPLC peak present in good and absent in poor durum varieties

Variety	Retention time (min)	Total peak area (%)
DWR 2006	37.05	18.6
MACS 1967	36.00	23.2
MACS 2694	36.74	20.3
PDW 215	36.45	14.6
PDW 274	NF**	-
WH 896	37.05	17.2
Lira 42*	NF	-
Lira 45*	37.17	26.2

\*Italian durum wheat

\*\* Not found

#### 4.2.7. Conclusions

Assessment of the physical characteristics of durum varieties showed that those having the highest test weight did not necessarily give the highest semolina yield. MACS 1967 that had significantly highest protein content produced good quality spaghetti in terms of firmness, low cooking loss, and stickiness. On the other hand, variety PDW 215 which had significantly low protein content also produced good spaghetti. Otherwise, the above two varieties were not similar in any of the physical or chemical properties. Hence, high protein content need not necessarily be responsible for optimum cooking quality of spaghetti. It is probable that along with protein, starch also would have influenced the quality of spaghetti. Variety PDW 274 which had the lowest amount of wet gluten and acetic acid insoluble protein content, showed poor spaghetti quality. Biochemical studies also showed that this variety lacked in a 45 kD polypeptide and a specific peak (GliPK36-37) in RP-HPLC. However, albumin and globulin fractions of good and poor durum varieties had almost similar pattern in SDS-PAGE, whereas significant differences were observed in gliadin and globulin fractions of these varieties. Scanning electron micrograph of PDW 274 variety was also slightly different from the other varieties. Variety WH 896, in spite of having good results for spaghetti firmness and stickiness after 10 min cooking, could not maintain its wholesome appearance, especially its firmness, for longer time. This could be because of its higher starch content as a result of lower protein content. This variety, due to its lower kernel hardness, had resulted in lower semolina yield with higher percentage of flour fraction. On the other hand, WH 896 had significantly higher yellow pigment resulting in spaghetti with higher yellowness. It can be contemplated that if the protein content of this variety



were to be increased, either at breeding stage or during the stage of processing, it would probably become more suitable for spaghetti preparation. The results showed that Indian durum varieties compared well, in one property or the other, in their physicochemical, biochemical, semolina milling and spaghetti making properties, with some of the well-known Canadian and Italian durum varieties that have been reported in literature.

### **4.3.1. Introduction**

Pasta quality, especially its structural and textural properties, is influenced by several factors, the most important being the quality of the raw material used and the drying conditions employed. Drying is considered as the most difficult and critical step in pasta production (Banasik, 1981; Zweifel et al., 2000). The objective of drying is to lower the moisture content of the product from 30-34% to around 12% so that the pasta will be translucent, intact in shape, and store without shattering. When the drying is too slow, pasta products tend to spoil or become moldy, and when the drying is too rapid, moisture gradients occur that causes the product to crack or check. This can occur during the drying period or afterwards, even after pasta has been packaged and sold (Aktan and Khan, 1992).

Two drying processes known for processing of pasta are: low temperature (LT) or conventional drying which refers to the use of temperatures no higher than 60 °C (Dalbon and Oehler, 1983; Pollini, 1998), and high temperature (HT) drying which refers to temperatures between 60-100 °C (Zweifel et al., 2003). However, there is no defined and specific temperature range for the drying processes. For example, Pollini (1998) has reported 60-84 °C for HT and above 84 °C for very high temperature (VHT) drying, whereas Malcolmson et al (1993) reported temperatures greater than 90 °C for VHT process. The main advantages of HT drying are increased microbiological safety, higher productivity, and improved textural properties of pasta (Zweifel et al., 2003).

In the past 20 years, LT drying has been gradually replaced with HT drying (Zweifel et al., 2003). However, LT drying process is still a useful technique for research studies, because at lower conventional temperatures, cooking quality largely depends on the intrinsic characteristics of the raw materials (Cubadda, 1986). However, the HT drying technique seems to modify the semolina properties and enhance cooking quality independent of semolina quality (Resmini and Pagani, 1983; Cubadda, 1985).

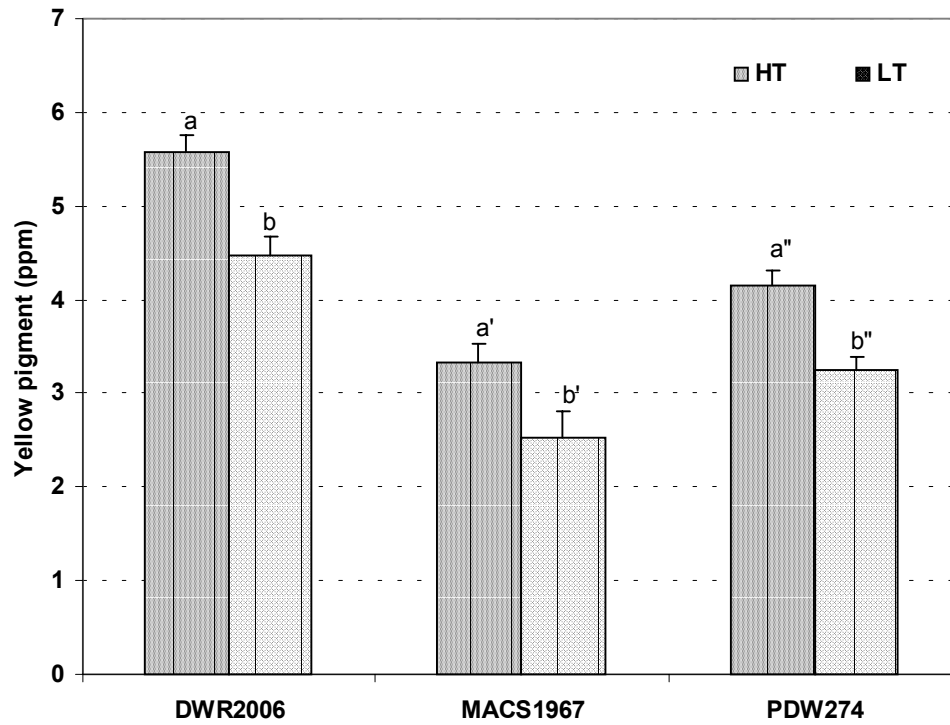
Overall quality of durum wheat pasta is influenced primarily by the properties of the protein and the starch fraction, and their transformation during extrusion, drying, and cooking (Zweifel et al., 2000). Pasta cooking quality has also been described as a competition between starch gelatinization and protein network formation (Dalbon et al., 1982; Resmini and Pagani, 1983). Thus, it is possible that starch properties also contribute to pasta cooking quality. Although starch represents up to 80% of semolina dry matter (Fortini, 1988) and is the major component of pasta, it has received less attention in research (Lintas and D'Appolonia, 1973; Grant et al., 1993; Vansteelandt and Delcour, 1998; Guler et al., 2002).

As discussed in the previous chapter, among the six Indian durum varieties studied for their cooking quality, varieties DWR 2006 and MACS 1967 had good spaghetti making properties while the variety PDW 274 had poor spaghetti making properties. In the present study, semolina from these three Indian durum wheat varieties, namely, DWR 2006, MACS 1967, and PDW 274 were processed into spaghetti following HT drying (85 °C) method.

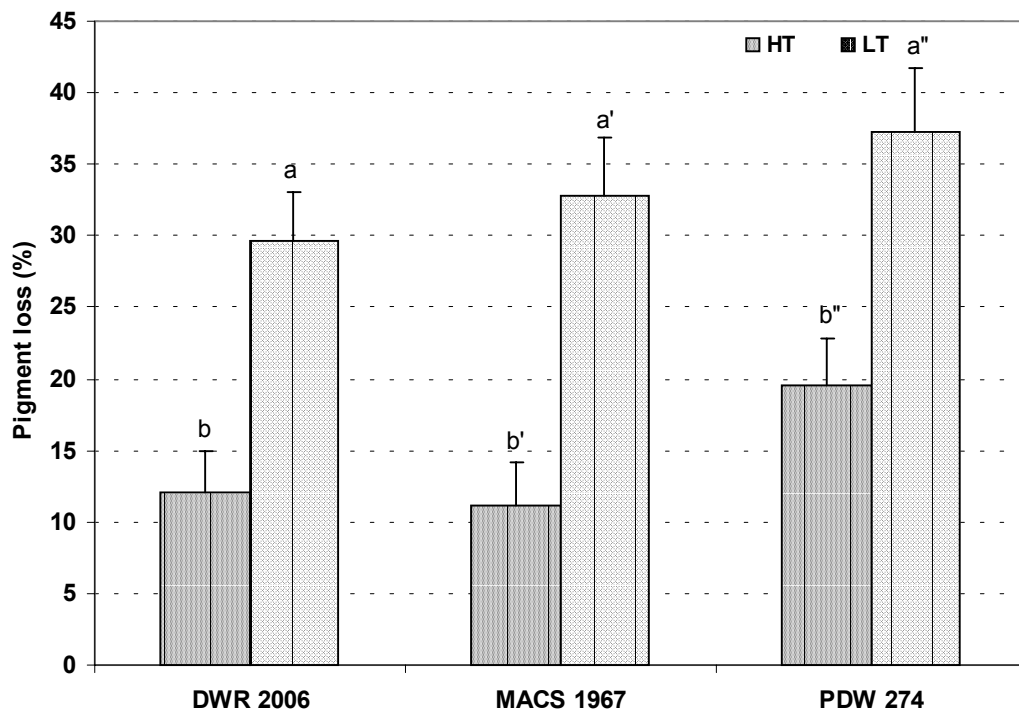
Effect of HT drying on spaghetti quality and its cooking quality were studied. These results were compared with those of LT drying process discussed in the previous chapter. Cooking quality of spaghetti from MACS 1967 (good quality) and PDW 274 (poor quality) was also studied during different cooking time intervals. Starch pasting properties using micro visco-amylograph, and also microstructure of samples were evaluated to study the behavior of starch and protein during spaghetti processing and cooking, especially to compare the results between the two durum varieties having good and poor spaghetti making properties, respectively.

#### **4.3.2. Yellow pigment content and color characteristics of spaghetti**

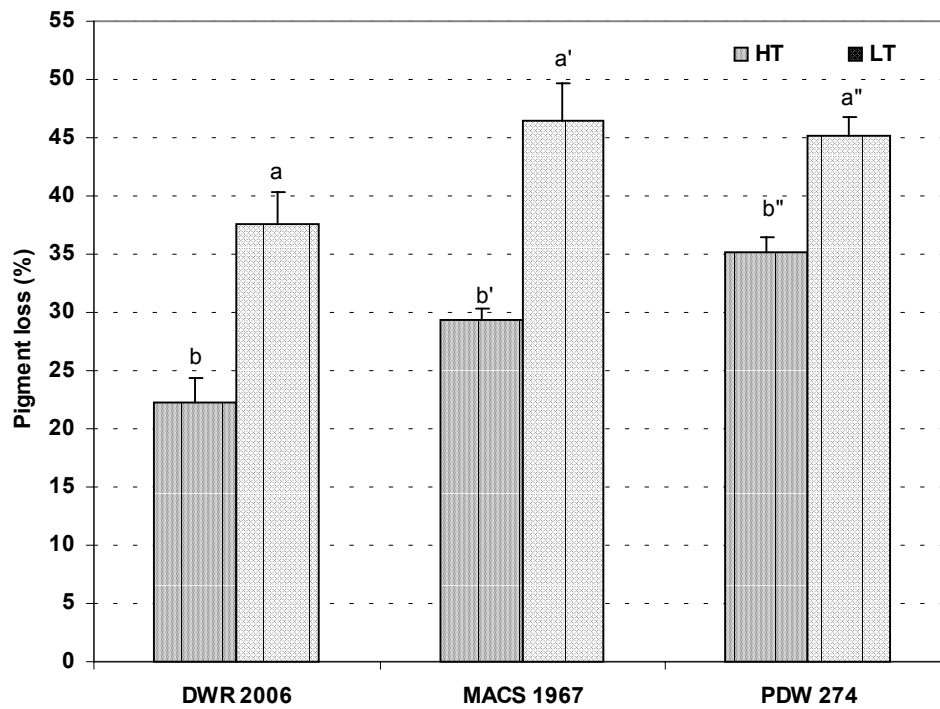
Yellow pigment content of spaghetti from three Indian durum wheats prepared by HT (85 °C) and LT (55 °C) drying processes are shown in Fig. 47. Yellow pigment content in semolina from their respective wheat varieties has been reported in Chapter 2 (Table 16). Significant ( $p < 0.05$ ) differences were found in the yellow pigment content of spaghetti samples dried at LT and HT for each variety. Accordingly, Fig. 48 shows loss in yellow pigment content during the processing of semolina into spaghetti under LT and HT drying conditions. Pigment loss in LT-dried spaghetti samples ranged from 29.6% (DWR 2006) to 37.2% (PDW 274), whereas variety PDW 274 had the maximum pigment loss of 19.5% following HT drying processing. There were also no significant differences in pigment loss either by HT or LT processing between spaghetti samples from DWR 2006 and MACS 1967. Fig. 49 shows total yellow pigment loss due to milling and spaghetti processing. These data



**Fig. 47.** Effect of drying temperature on yellow pigment content of spaghetti. HT, High temperature; LT, Low temperature. Data are expressed as mean  $\pm$ SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.



**Fig. 48.** Yellow pigment loss during the processing of semolina to spaghetti dried at HT and LT. HT, High temperature; LT, Low temperature. Data are expressed as mean  $\pm$ SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.



**Fig. 49.** Cumulative pigment loss during the processing of durum wheat into spaghetti dried at HT and LT. HT, High temperature; LT, Low temperature. Data are expressed as mean  $\pm$ SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

indicate that drying process is the main responsible factor for pigment loss of spaghetti, although some part of pigment loss might also occur during mixing and extrusion processes. Earlier, Dexter et al (1981) showed that 75% of pigment loss occurred during LT drying process. Previous studies have linked the loss of pigment during spaghetti processing to lipoxygenase (LOX) bleaching activity in the semolina (Irvine and Anderson, 1953; McDonald, 1979). Activity of endogenous enzymes, especially oxidative enzymes (lipoxygenases, peroxidases, and polyphenol oxidases), starts immediately after addition of water to semolina and the presence of oxygen is necessary for their activity (Dalbon et al., 1998). In the present study, even though the durum variety MACS 1967 had significantly high LOX activity (Ch. 1A, Fig. 25) the spaghetti produced from it following HT drying process showed the lowest pigment loss comparable ( $p < 0.05$ ) to that of spaghetti from DWR 2006 with a higher pigment content and a significantly lower LOX activity. This can be due to the partial inactivation of endogenous LOX by HT drying process which is in accordance with previous reports (Dexter et al., 1981; Acquistucci, 2000; Zweifel et al., 2003).

Color characteristics of spaghetti dried at LT and HT are reported in Table 24. Both brightness (L value) and yellowness (b value) of spaghetti samples processed at HT were significantly ( $p < 0.05$ ) higher than those of corresponding LT-dried spaghetti samples. It is well known that yellowness and brownness ( $100-L$ ) are correlated both to amount of pigment present and to enzymatic reactions (Oliver et al., 1993). Some phenolase and oxidase enzymes responsible for browning and pigment loss of spaghetti can be



**Table 24.** Effect of drying temperatures (LT and HT)\* on color characteristics\*\* of spaghetti

Variety	LT		HT	
	L	b	L	b
<b>DWR 2006</b>	53.90 ± 0.34 <sup>bl</sup>	21.95 ± 0.20 <sup>b<sup>1</sup></sup>	57.29 ± 0.19 <sup>al</sup>	23.71 ± 0.16 <sup>a<sup>1</sup></sup>
<b>MACS 1967</b>	52.55 ± 0.22 <sup>bm</sup>	19.41 ± 0.28 <sup>b<sup>m</sup></sup>	55.55 ± 0.46 <sup>am</sup>	21.62 ± 0.25 <sup>a<sup>n</sup></sup>
<b>PDW 274</b>	54.66 ± 0.18 <sup>bl</sup>	20.15 ± 0.22 <sup>b<sup>m</sup></sup>	55.68 ± 0.28 <sup>am</sup>	22.55 ± 0.06 <sup>a<sup>m</sup></sup>

Data are expressed as mean ±SD. Values followed by different letters l,m..., in columns (comparison between varieties) and a,b..., in rows (comparison between drying conditions), differ significantly (p<0.05).

\*\* HT, High temperature; LT, Low temperature

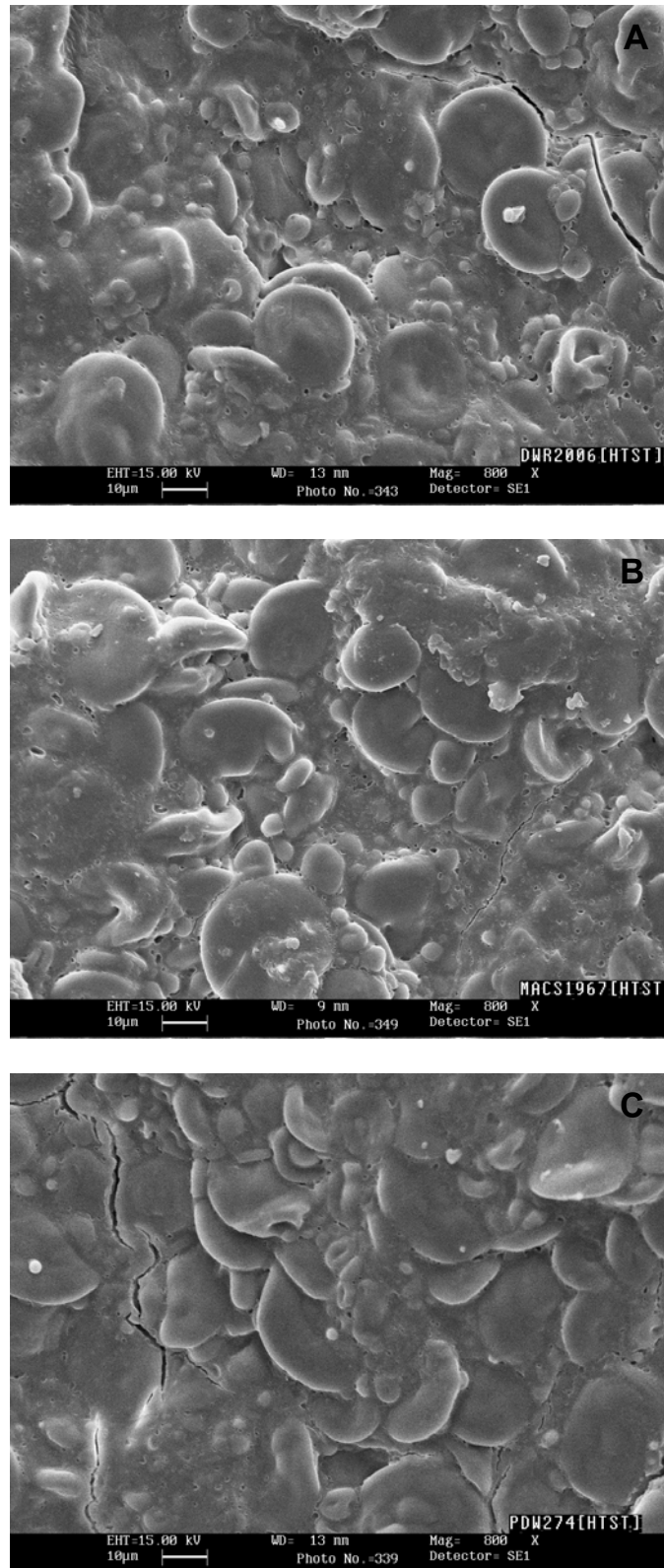
\* L, Brightness; b, Yellowness

inactivated during HT drying (Pollini, 1998). Results of the present study agree well with literature data (De Stefanis and Sgrulletta, 1990; Acquistucci, 2000; Zweifel et al., 2003) and confirm that HT-dried spaghetti has higher yellowness value, most probably due to partial inactivation of lipoxygenase.

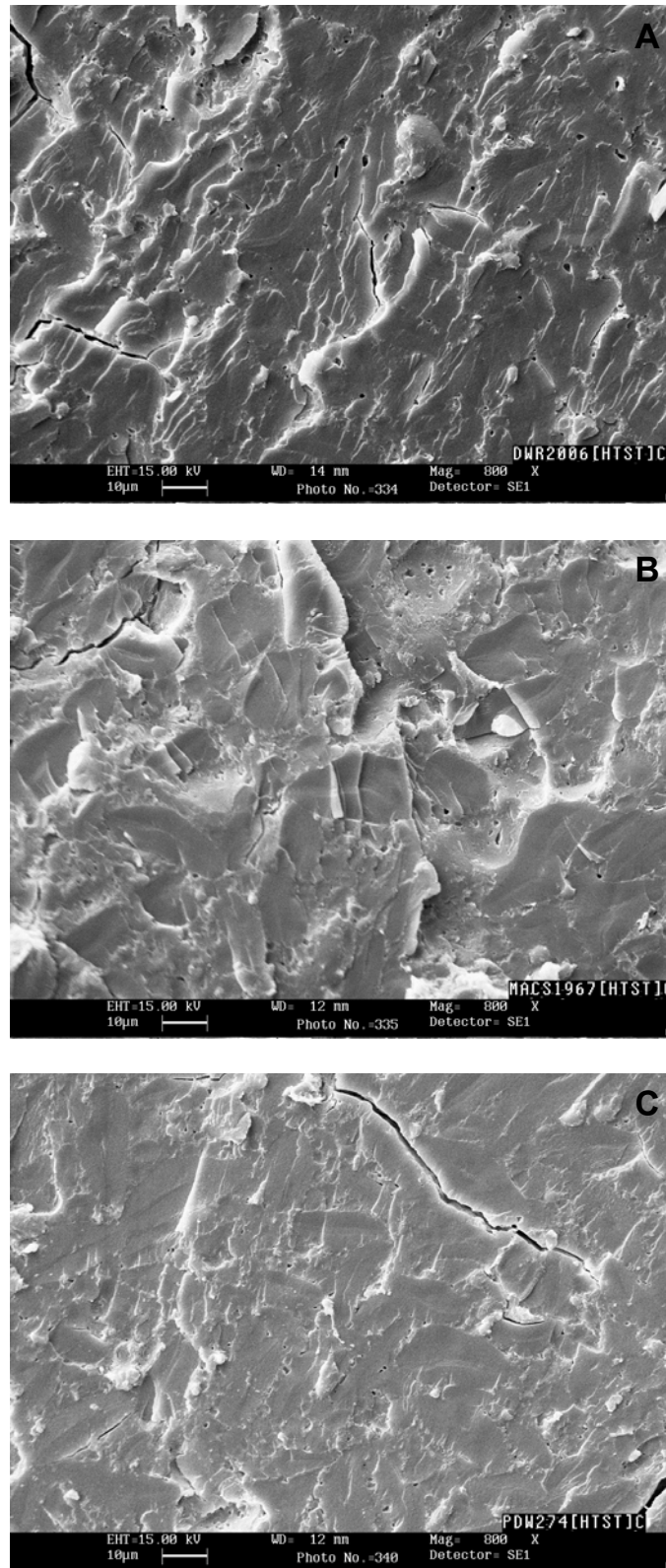
#### **4.3.3. Microstructure of raw spaghetti**

Scanning electron micrographs of surface and cross-section of spaghetti processed by HT drying from three Indian durum wheat varieties namely, DWR 2006, MACS 1967, and PDW 274 are shown in Fig. 50 and Fig. 51, respectively. HT-dried spaghetti from all three durum varieties showed a smoother surface with a more continuous protein matrix surrounding the starch granules. Interestingly, no significant differences were observed between the surface SEM images of these three HT-dried spaghetti samples. On the other hand, as it was previously shown (Ch. 2, Fig. 42) the surface microstructure of LT-dried spaghetti from PDW 274 (poor durum variety) was quite different from those of good durum varieties (DWR 2006 and MACS 1967).

These observations indicate that exposure of spaghetti strands to high temperatures for a short time probably results in a more pronounced gelatinization of the starch granules resulting in an apparent smooth surface. On the other hand, exposure of spaghetti to low temperatures even though for a long time might not be sufficient for pronounced gelatinization of starch to take place. However, in the absence of sufficient water, extensive gelatinization of starch is not possible in both the drying conditions.



**Fig. 50.** Scanning electron micrographs of surface of spaghetti samples dried at high temperature. (A) DWR 2006; (B) MACS 1967; (C) PDW 274.



**Fig. 51.** Scanning electron micrographs of cross-section of spaghetti samples dried at high temperature. (A) DWR 2006; (B) MACS 1967; (C) PDW 274.

As seen in Fig. 50, some cracks, like those present in LT-dried spaghetti, are also observed in the HT-dried spaghetti. This might be partly due to shrinkage during sample preparation and partly due to tension in spaghetti dough during drying (Cunin et al., 1995). However, the number of holes and gaps between starch granules is very few or absent when compared to those of LT-dried spaghetti.

There are very few reports available regarding the study of microstructure of spaghetti dried at HT and LT (Resmini and Pagani, 1983; Zweifel et al., 2003). Studies on protein solubility have demonstrated that high-temperature drying denatures protein extensively (Cubadda, 1989; De Stefanis and Sgrulletta, 1990; Aktan and Khan, 1992). Resmini and Pagani (1983) reported that proteins were polymerized by high drying temperatures and that starch granules appeared embedded within a protein matrix. On the other hand, Güler et al (2002) reported that 20% of starch granules are partially or completely gelatinized during HT drying. Zweifel et al (2003) demonstrated that slight swelling of the starch granules and denaturation of surface proteins of starch granules occur due to HT drying process. Therefore, it may be possible that smooth and compact surface structure of HT-dried spaghetti samples which is significantly different from that observed in LT-dried spaghetti, is due to protein denaturation and slight starch gelatinization.

The micrographs of cross-section of spaghetti dried at 85 °C (Fig. 51) showed a compact structure comprising of starch granules surrounded by

protein matrix. Fracture of hard spaghetti strands for electron microscopy studies resulted in a shining cross section in which all starch granules are visible in a broken form embedded in protein matrix. In contrast to surface microstructure, cross-section structure of HT-dried spaghetti samples was not significantly different from their corresponding LT-dried spaghetti reported in Fig. 42 (Ch. 2), although HT-dried spaghetti appeared to be somewhat more compact and dense.

#### **4.3.4. Starch pasting properties**

Starch pasting properties of freeze dried samples of mixed dough and extruded dough, and LT- and HT-dried spaghetti samples, all from the three Indian durum varieties were carried out using micro visco-amylgraph to study the changes that would have taken place in the starch fraction during processing. Pasting properties of semolina from these varieties have already been reported in Ch. 2 (Table 19). Relationships between starch pasting properties and spaghetti cooking quality are also discussed in this section.

Results of pasting properties of spaghetti dough before and after extrusion are shown in Table 25. Result shows an increase in the onset gelatinization temperatures of mixed dough from varieties DWR 2006 and PDW 274 compared to those of their corresponding semolina samples. On the other hand, there was no significant difference between onset gelatinization temperatures of dough and semolina of MACS 1967. Results also showed that there was no significant difference in the onset gelatinization temperatures of dough before and after extrusion in all the three samples.

**Table 25.** Starch pasting properties of spaghetti dough before and after extrusion

	Onset gelatinization Temp.(°C)	Peak viscosity (BU)	Breakdown viscosity (BU)	Final viscosity (BU)	Setback viscosity (BU)
<b>DWR 2006</b>					
Mixed dough	66.7 <sup>a</sup>	833.5 <sup>a</sup>	110 <sup>b</sup>	1197 <sup>a</sup>	455 <sup>b</sup> (38)*
Extruded dough	67.5 <sup>a</sup>	777.0 <sup>b</sup>	131 <sup>a</sup>	1129 <sup>b</sup>	478 <sup>a</sup> (42)
<b>MACS 1967</b>					
Mixed dough	66.5 <sup>a'</sup>	830.5 <sup>a'</sup>	117 <sup>a'</sup>	1186 <sup>a'</sup>	511 <sup>a'</sup> (43)
Extruded dough	66.9 <sup>a'</sup>	795 <sup>b'</sup>	125 <sup>a'</sup>	1131 <sup>b'</sup>	497 <sup>a'</sup> (44)
<b>PDW 274</b>					
Mixed dough	65.6 <sup>a''</sup>	735.0 <sup>a''</sup>	96 <sup>a''</sup>	1120 <sup>a''</sup>	593 <sup>a''</sup> (53)
Extruded dough	65.8 <sup>a''</sup>	681.0 <sup>b''</sup>	103 <sup>a''</sup>	1087 <sup>b''</sup>	492 <sup>b''</sup> (45)

Data are expressed as mean of duplicate analysis. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

\* Values in parentheses are setback as a percentage of final viscosity.

There was no change in peak viscosity values due to mixing process, except in PDW 274 sample that showed a marginal decrease compared to that of its corresponding semolina sample. The onset gelatinization temperature of extruded dough from all varieties was significantly lower than their corresponding mixed dough. Even though the maximum temperature attained during dough extrusion was only between 40-42 °C, decrease in peak viscosity could be due to damage to the starch granules incurred during the extrusion process.

The results showed that both mixing and extrusion processes increased the breakdown viscosity compared to those of respective semolina samples, and the increase in breakdown viscosity due to extrusion was more significant. On the other hand, the mixing and extrusion processes decreased the final viscosity values significantly compared to those of respective semolina samples. The changes taking place in the amylograph characteristics of the dough after extrusion can perhaps be attributed to mechanical damage of starch taking place during the process. Presence of damaged starch results in decreased swelling and hydration of the granule resulting in reduced pasting viscosity. In this context, Lintas and D'Appolonia (1973) had reported that some mechanical damage to starch occur during mixing and extrusion operations. Banasik et al., (1976) also noticed that the extrusion process promotes a partial loss of starch granule structure. Results of the present study showed that semolina from durum variety MACS 1967 showed the least variation in the pasting parameters due to mixing and extrusion. Morris et al (1997) and Batey (2000) have explained that



interactions between starch and protein, as well as a contribution from the protein itself, affect pasting viscosity and properties. In this context, it is possible that significantly higher protein content of MACS 1967, compared to the other two varieties, and its interactions with starch, might have resulted in least variation in its pasting properties due to processing conditions.

Visco-amylograms of spaghetti from DWR 2006, MACS 1967 and PDW 274 dried at LT and HT conditions are shown in Fig. 52. Data for starch pasting properties of spaghetti samples are summarized in Table 26. The onset gelatinization temperature of LT- and HT-dried spaghetti from three Indian durum varieties was significantly higher than those of their corresponding extruded dough and semolina. Zweifel et al (2000) and Güler et al (2002) studying starch properties of semolina and pasta using Differential Scanning Calorimeter found that onset gelatinization temperature of pasta was significantly higher than that of corresponding semolina.

Peak viscosity of both LT- and HT-dried spaghetti was more than that of corresponding extruded dough. However, there was no significant difference ( $p < 0.05$ ) between peak viscosity of LT-dried spaghetti and corresponding semolina. Peak viscosity of HT-dried spaghetti was significantly lower than that of corresponding semolina and LT-dried spaghetti. Though the extrusion process significantly increased the breakdown viscosity compared to that of semolina, both the drying processes, on the other hand, significantly decreased the breakdown viscosity. Breakdown viscosity of LT-dried spaghetti was however, significantly higher than that of corresponding

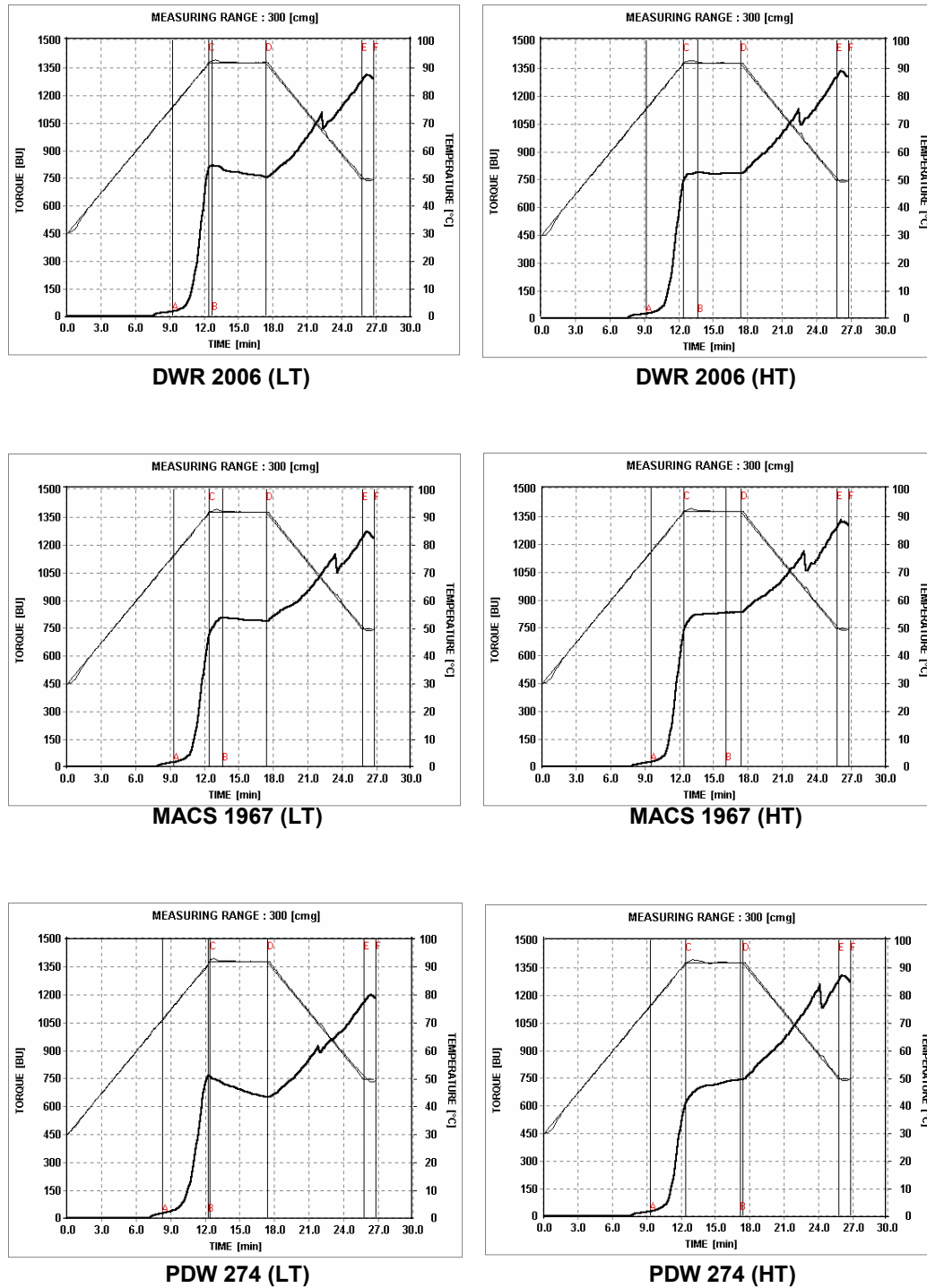


Fig. 52. Micro visco-amylograms of LT and HT-dried spaghetti from three Indian durum wheat varieties

**Table 26.** Starch pasting properties of raw spaghetti dried at LT and HT \*

	Onset gelatinization Temp. (°C)	Peak viscosity (BU)	Breakdown viscosity (BU)	Final viscosity (BU)	Setback viscosity (BU)
<b>DWR 2006</b>					
LT	75.9 <sup>a</sup>	821.5 <sup>a</sup>	63 <sup>a</sup>	1293.5 <sup>a</sup>	520.5 <sup>a</sup> (40)**
HT	75.4 <sup>a</sup>	791.5 <sup>b</sup>	7.0 <sup>b</sup>	1304.5 <sup>a</sup>	518.5 <sup>a</sup> (40)
<b>MACS 1967</b>					
LT	76.1 <sup>a'</sup>	833 <sup>a'</sup>	16.5 <sup>a'</sup>	1237.0 <sup>b'</sup>	450.5 <sup>a'</sup> (36)
HT	77.2 <sup>a'</sup>	807 <sup>b'</sup>	0.0 <sup>b'</sup>	1302.5 <sup>a'</sup>	460.0 <sup>a'</sup> (35)
<b>PDW 274</b>					
LT	70.8 <sup>b''</sup>	760.0 <sup>a''</sup>	112.5 <sup>a''</sup>	1181.5 <sup>b''</sup>	504 <sup>b''</sup> (42)
HT	76.3 <sup>a''</sup>	744.5 <sup>b''</sup>	0.0 <sup>b''</sup>	1272.0 <sup>a''</sup>	537 <sup>a''</sup> (42)

Data are expressed as mean of duplicate analysis. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

\* LT, Low temperature; HT, High temperature

\*\* Values in parentheses are setback as a percentage of final viscosity.

HT-dried spaghetti. Breakdown viscosity of semolina, mixed dough, and extruded dough of PDW 274 was less than those of DWR 2006 and MACS 1967. However, LT-dried spaghetti from PDW 274 had higher breakdown viscosity than LT-dried spaghetti of DWR 2006 and MACS 1967. In the case of final viscosity, DWR 2006 and MACS 1967 presented similar trend in that final viscosity of HT-dried spaghetti was higher than those of corresponding LT-dried spaghetti, extruded dough and semolina. Final viscosity of LT-dried spaghetti was also higher than that of semolina. However, final viscosity of HT-dried spaghetti from PDW 274 was higher than that of LT-dried spaghetti, but former was not different from that of its corresponding semolina, while latter was less than that of its semolina. Similarly, there was no significant difference between setback viscosity of HT-dried spaghetti from PDW 274 and its corresponding semolina. On the other hand, setback viscosity as a percentage of the final viscosity of both HT- and LT-dried spaghetti from PDW 274 was similar to that of its corresponding semolina (42%), whereas there was a decrease in this value for HT- and LT-dried spaghetti from DWR 2006 and MACS 1967.

Lintas and D'Appolonia (1973) showed that semolina starch had a higher peak viscosity than the starch isolated from LT-dried spaghetti. They pointed out that a certain amount of starch was damaged during processing but the greatest change occurred during the drying step when amyolytic enzymes would act on mechanically damaged starch. In the present study, insignificant difference between peak viscosity of LT-dried spaghetti samples and their corresponding semolina samples might partly be attributed to low

amylolytic activity in semolina as measured using the falling number apparatus. Lower peak viscosity of HT-dried pasta can be attributed to slight gelatinization of starch taking place during drying process (Güler et al., 2002; Zweifel et al., 2003). Yue et al., (1999) using Rapid Visco-Analyser (RVA) also reported that peak viscosity of starch isolated from LT (40 °C) or HT (70 °C) spaghetti was less than that of their corresponding semolina.

Increase in peak viscosity of LT and HT-dried spaghetti compared to cold extruded dough could be explained by findings of Hoover and Vasanthan (1994) from their study on heat-moisture treated wheat starches. According to them, in native state of starch, small starch crystallite regions are destabilized in amorphous regions, particularly by interactions between amylose chains; and amylose and outer branches of amylopectin molecules. Later, application of heat treatment (pasta drying) may reduce this destabilization resulting in increased peak viscosity. Zweifel et al (2000) also reported the stabilization of starch granules during the drying process.

The stability of hot starch pastes is described by breakdown viscosity, higher values indicating lower stability. In the present study, HT-dried spaghetti exhibited lower breakdown viscosity than LT-dried spaghetti. This can once again be attributed to changes the starch molecules would have undergone due to high temperature drying.

The lower final viscosity of LT-dried spaghetti samples was probably due to amylose-lipid complex formation that may have restricted

recrystallization of amylose during cooling (Yue et al., 1999; Güler et al., 2002). In conclusion, there is still a lack of a general agreement among researchers to show the relationship between starch properties, especially pasting properties and pasta quality. Yue (1997) could not find any significant correlation among starch properties and pasta quality, confirming that protein was the primary factor affecting pasta quality. This is also confirmed by Sissons and Batey (2003). On the other hand, a number of other research workers have established relationships between starch properties and quality of different types of noodles where starch tends to play a more important role (Crosbie, 1991; Konik et al., 1992; 1993; 1994; Panozzo and Mc Cormick, 1993).

#### **4.3.5. Distribution and solubility of protein fractions**

Results of modified Osborne protein fractionation of semolina from the three Indian durum wheat varieties, namely, DWR 2006, MACS 1967, and PDW 274 and their respective spaghetti samples dried at both LT and HT are shown in Table 27. Protein fractionation of semolina from these varieties showed that gliadin (70% ethanol extractable) fraction constituted the highest amount of protein among the fractions. This was followed by residue protein (insoluble), albumin + globulin (soluble in 0.15 M NaCl) and glutenin (soluble in 0.1 M acetic acid) fractions. Same trend was seen in all the three varieties. However, the amount of albumin + globulin and also residue protein fraction was significantly different among the varieties. Earlier, Dexter and Matsuo (1977a) reported an average of 16% and 12% for albumin + globulin and glutenin fractions, respectively for semolina from some Canadian durum

**Table 27.** Effect of drying temperature\* on solubility of protein fractions of spaghetti

	Fractions (%)**			
	Albumin + Globulin	Gliadin	Glutenin	Residue
<b>DWR 2006</b>				
Semolina	19.92 <sup>c</sup>	41.08 <sup>ab</sup>	9.27 <sup>a</sup>	27.73 <sup>e</sup>
LT Spaghetti	16.33 <sup>e</sup>	40.13 <sup>b</sup>	7.34 <sup>b</sup>	31.30 <sup>d</sup>
HT Spaghetti	9.07 <sup>h</sup>	30.40 <sup>d</sup>	3.62 <sup>c</sup>	52.91 <sup>b</sup>
<b>MACS 1967</b>				
Semolina	21.11 <sup>b</sup>	39.87 <sup>bc</sup>	8.54 <sup>a</sup>	26.50 <sup>f</sup>
LT Spaghetti	18.16 <sup>d</sup>	38.68 <sup>c</sup>	6.47 <sup>b</sup>	32.17 <sup>d</sup>
HT Spaghetti	9.92 <sup>g</sup>	27.91 <sup>e</sup>	3.08 <sup>c</sup>	55.59 <sup>a</sup>
<b>PDW 274</b>				
Semolina	23.30 <sup>a</sup>	42.19 <sup>a</sup>	8.57 <sup>a</sup>	22.74 <sup>g</sup>
LT Spaghetti	19.57 <sup>c</sup>	40.86 <sup>b</sup>	6.63 <sup>b</sup>	27.34 <sup>ef</sup>
HT Spaghetti	11.18 <sup>f</sup>	30.80 <sup>d</sup>	3.60 <sup>c</sup>	50.02 <sup>c</sup>

Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

\*LT, Low temperature; HT, High temperature.

\*\* Data are mean of two values expressed as percentage of total protein of semolina.

varieties. In another study, Wasik (1978) reported lower values for albumin + globulin and higher values for glutenin fractions for certain Canadian durum varieties. Chen and Bushuk (1970) found that 15-20% of the total protein was comprised of albumins and globulins, 40-50% of gliadin, 12-20% of glutenin and 17-35% of insoluble residue. However, in the present study albumins and globulins was slightly higher and glutenin was lower than that of above mentioned range. In other words, glutenin : gliadin ratio in Indian durum wheats seems to be less than that of Canadian varieties. On the other hand, percentage of albumins + globulins and gliadin was significantly higher, while residue protein content was significantly lower in PDW 274 (poor variety) compared to other two varieties. Spaghetti drying at both low temperature (55 °C) and high temperature (85 °C) showed marked effect on the solubility of different protein fractions. Gliadin fraction was less affected by drying processes, particularly the LT drying. Wasik (1978) also showed that conventional drying of pasta (39 °C) did not affect the solubility of gliadin fraction. Pence et al (1953) studying the effect of heat treatment on gluten, found that the gliadin fraction was the most stable to heat denaturation, as measured by acetic acid solubility test. In contrast, Ummadi et al (1995) found that extrusion processing at 50 °C or 96 °C markedly decreased the solubility of all protein fractions especially gliadin. Aktan and Khan (1992) concluded that the gliadin fraction is essentially not affected by high-temperature drying of pasta.

As seen in Table 27, significantly highest amount of residue protein was observed in HT-dried spaghetti from MACS 1967, whereas both LT- and



HT-dried spaghetti from PDW 274 showed a significantly lower amounts of residue protein.

Reduction in solubility of proteins due to high temperatures may be a result of protein-starch interactions (Resmini and Pagani, 1983) or due to polymerization of proteins (through disulfide linkages) (Ummadi et al., 1995). Whatever the reasons are for insolubilization of protein fractions, the ability of gluten proteins to aggregate during heating differs markedly accordingly to the type and quality of wheat. Therefore, the capacity for proteins to associate into insoluble complexes and possibly to form an insoluble network is characteristic of high quality aestivum wheat for baking and high quality durum wheat for pasta-making (Jeanjean et al., 1980).

#### **4.3.6. Spaghetti cooking quality**

Cooking quality characteristics of three spaghetti samples dried at high temperature conditions and cooked for 10 min are shown in Table 28. Comparing these results with cooking quality data of spaghetti samples dried at LT (Table 20, Ch. 2), it can be seen that HT drying has significantly decreased the cooking loss values. This is consistent with previous reports (Dexter et al., 1990; Grant et al., 1993; Wyland and D'Appolonia, 1982). Results showed that cooking loss of HT-dried spaghetti from weak variety PDW 274 decreased considerably compared to the other two varieties. Accordingly, while a 14% decrease was observed for PDW 274, decrease of 6 and 9% was observed for DWR 2006 and MACS 1967, respectively.

**Table 28.** Cooking quality characteristics of spaghetti dried at HT after 10 min cooking

Variety	Cooking loss (%)	Cooked weight (g)	Firmness (gf)	Stickiness (N/m <sup>2</sup> )
DWR 2006	6.21 ± 0.06 <sup>b</sup>	27.2 ± 0.21 <sup>b</sup>	83.4 ± 4.3 <sup>b</sup>	502.5 ± 6.4 <sup>b</sup>
MACS 1967	5.26 ± 0.10 <sup>c</sup>	27.3 ± 0.20 <sup>b</sup>	97.8 ± 5.6 <sup>a</sup>	465.0 ± 8.3 <sup>c</sup>
PDW 274	7.01 ± 0.08 <sup>a</sup>	29.9 ± 0.15 <sup>a</sup>	76.0 ± 2.4 <sup>b</sup>	666.2 ± 10.2 <sup>a</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

The reason for direct effect of HT drying on improvement of cooking loss is not clear. As was discussed earlier, changes in proteins, starch and microstructure of spaghetti due to HT drying process might have contributed to this improvement in cooking loss. Studies on spaghetti microstructure, starch pasting properties, and solubility of protein fractions showed that protein aggregation and denaturation was much pronounced under HT drying conditions. This was accompanied with slight starch gelatinization as well. Therefore, coagulated and strengthened protein film on the surface of HT-dried spaghetti might have been able to prevent loss of gelatinized starch during cooking resulting in lowered cooking loss (Jeanjean et al., 1980; Dexter et al., 1983).

The cooked weight values of HT-dried spaghetti samples were less than those of LT-dried spaghetti, however, this decrease was not significant ( $p < 0.05$ ). Cooked weight of HT-dried spaghetti from PDW 274 (poor variety) was significantly higher than those of DWR 2006 and MACS 1967, similar to the observation made in LT-dried spaghetti (Ch. 2). Decrease in cooked weight of HT-dried spaghetti could be due to the reduction in water uptake of spaghetti strands because of the heat strengthened protein network on the surface of spaghetti. This is in agreement with Wyland and D'Appolonia (1982) and Zweifel et al (2003).

Drying of spaghetti at HT conditions significantly ( $p < 0.05$ ) increased the firmness of all spaghetti samples. This is in agreement with earlier reports (Aktan and Khan, 1992; Zweifel et al., 2003). Similar to cooking loss, firmness

of spaghetti from poor variety PDW 274 improved considerably, more than those of other two varieties due to HT drying. There was no significant difference between firmness value of spaghetti from PDW 274 and that of DWR 2006. HT drying process increased the firmness of spaghetti from PDW 274 by 45% compared to LT-dried spaghetti firmness, whereas this increase was 28% and 16% in case of DWR 2006 and MACS 1967, respectively. As was shown earlier (Ch. 2), variety PDW 274 had sufficient amount of protein for producing a good quality spaghetti but quality of its protein was not suitable. It can be concluded that while quality of protein was important for cooking quality of spaghetti dried at LT, quantity of protein and interactions between protein and starch had great effect on improving cooking quality, especially firmness of cooked spaghetti.

Sung and Stone (2003) indicated that coagulated gluten network plays an important role in imparting firmness to cooked pasta. As was shown earlier, solubility of gluten protein significantly decreased due to HT drying process probably due to its denaturation and polymerization. These changes in gluten molecule would have contributed to its strength during cooking. On the other hand, Dexter and Matsuo (1979b) pointed out that starch is the major component of semolina, and firmness in cooked spaghetti must, in part, be influenced by gelatinized starch properties. Cunin et al (1995) indicated potential interactions between coagulated protein and gelatinized starch components. The above observations were supported by scanning electron microscopy studies that showed less swollen starch granules in the core of HT-dried spaghetti compared to LT-dried spaghetti. This will be discussed in detail in the following sections.

In view of the above results, it is probable that improvement in firmness of cooked spaghetti from PDW 274 might be not only because of the strengthening of the gluten network but also due to an interaction between protein and starch that would have taken place under the HT drying conditions.

Another benefit of HT drying was a significant reduction in the stickiness of cooked spaghetti samples. This is in agreement with previous reports (De Stefanis and Sgrulletta, 1990; Grant et al., 1993; Zweifel et al., 2003). The stickiness is linked to the structural properties of pasta in the external part. Extensive starch swelling and amylose leaching has been reported to correlate well with a sticky pasta surface (Zweifel et al., 2003). Spaghetti stickiness is related to strand disintegration during cooking (Grant et al., 1993). Other authors have shown that the integrity of the external layer of pasta is very important in determining the amount of material that can be rinsed from cooked spaghetti, which correlates with stickiness of pasta (Dexter et al., 1985; D'Egidio et al., 1993). According to the above results and also previous results related to spaghetti microstructure and protein solubility, it can be contemplated that, in HT drying process a continuous and strong protein network which is formed on surface of spaghetti controls starch swelling and gelatinization during cooking. This would further prevent disintegration and consequently leaching of starch material to the surface leading to less sticky product.

Results of quality parameters of HT-dried spaghetti cooked for 20 min are shown in Table 29. Results showed an increase in cooking loss and

**Table 29.** Cooking quality characteristics of spaghetti dried at HT after 20 min cooking

Variety	Cooking loss (%)	Cooked weight (g)	Firmness (gf)
DWR 2006	8.12 ± 0.12 <sup>b</sup>	35.1 ± 0.31 <sup>b</sup>	67.5 ± 4.1 <sup>b</sup>
MACS 1967	7.51 ± 0.08 <sup>c</sup>	34.8 ± 0.24 <sup>b</sup>	75.2 ± 2.0 <sup>a</sup>
PDW 274	9.48 ± 0.15 <sup>a</sup>	38.5 ± 0.22 <sup>a</sup>	59.8 ± 1.3 <sup>c</sup>

Data are expressed as mean ± SD. Means of the same column followed by different letters are significantly different ( $p < 0.05$ ).

cooked weight and a decrease in firmness values as the duration of cooking time was increased. The cooked weight of spaghetti from poor variety PDW 274 was significantly higher than those of other two good varieties, indicating a softer product. It should be noted here that increase in cooking loss and decrease in firmness due to overcooking of HT-dried spaghetti samples was higher than those of LT-dried spaghetti. Increase in cooked weight due to overcooking was more or less similar (~ 29%) for all three HT-dried spaghetti samples, which was still lower than those of corresponding LT-dried spaghetti samples. Interestingly, firmness values of overcooked (20 min) HT-dried spaghetti samples were still higher and even comparable with those of LT-dried spaghetti cooked for 10 min. It is evident from the above results that changes in the protein molecules as well as the interactions between starch and proteins that would have taken place during HT drying was strong and very effective in maintaining the integrity of the spaghetti strands intact even after an extended period of cooking.

#### **4.3.7. Effect of cooking time on spaghetti quality**

To study the effect of cooking time on quality properties, HT- and LT-dried spaghetti from good variety MACS 1967 and poor variety PDW 274 were subjected to different cooking times varying from 4 to 20 min. In the present study, cooking of spaghetti for 10 min was considered optimum and beyond that was considered as overcooking. Results of this study are shown in Figures 53-58. As seen in Fig. 53, cooking loss of LT- and HT-dried spaghetti from MACS 1967 increased linearly from 4.35 to 7.78% and 3.6 to 7.72%, respectively. A strong relationship ( $r=0.98^{**}$ ) was found between

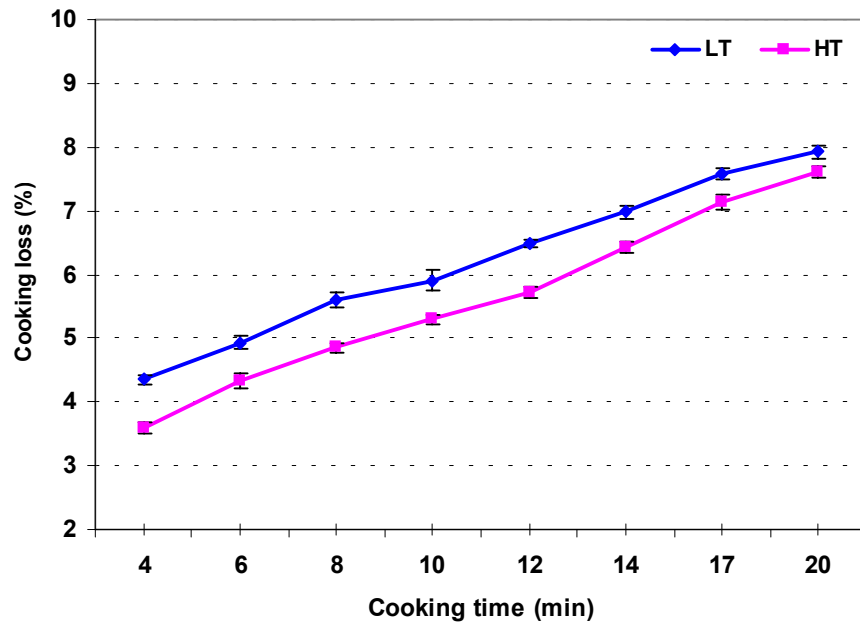


Fig. 53. Cooking loss of spaghetti from good variety MACS 1967 (dried at HT and LT) in different cooking times

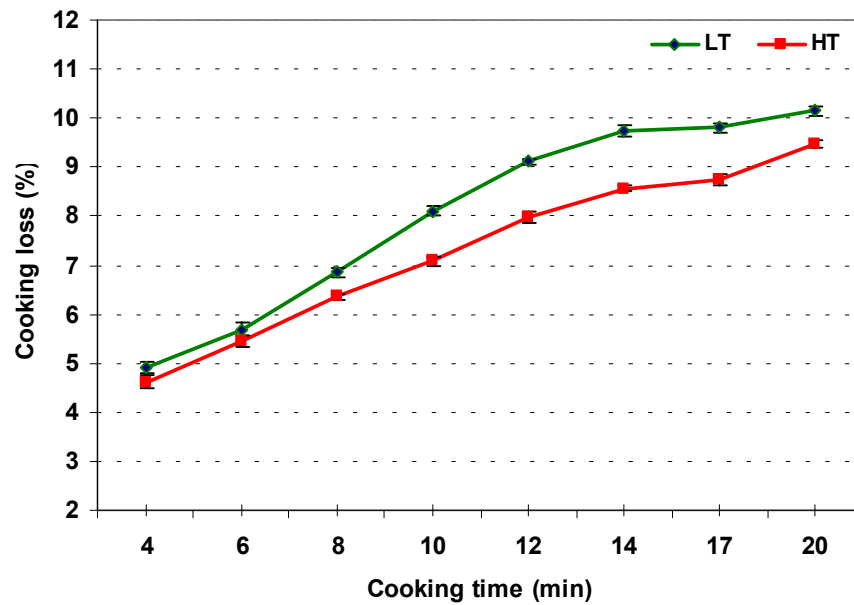


Fig. 54. Cooking loss of spaghetti from poor variety PDW 274 (dried at HT and LT) in different cooking times



different cooking times and cooking loss of LT-dried spaghetti. However, this correlation coefficient was slightly lower than that of HT-dried spaghetti ( $r=0.99^{**}$ ). Cooking loss of LT- and HT-dried spaghetti from PDW 274 linearly increased up to 14 min of cooking (Fig. 54). However, the rate of increase in cooking loss decreased after 14 min cooking. Both the spaghetti samples from poor variety PDW 274 had significantly ( $p<0.05$ ) higher cooking loss than good variety MACS 1967 at all the cooking times. Results showed that spaghetti samples from PDW 274 had lost most of its leaching material during the first 14 min of cooking. Whereas in the case of MACS 1967, there was a gradual increase in the cooking loss even up to 20 min of cooking, which shows a better cooking tolerance of spaghetti from MACS 1967. Cooking loss of LT- and HT-dried spaghetti samples from PDW 274 showed significant correlation with cooking times. However, correlation coefficient for LT-dried spaghetti ( $r=0.94^{**}$ ) was slightly lower than that of HT-dried spaghetti ( $r=0.97^{**}$ ).

Fig. 55 shows the linear increase in cooked weight of LT- and HT-dried spaghetti samples from variety MACS 1967 cooked for different time durations. A high correlation coefficient ( $r=0.99^{**}$ ) was found between cooking time and cooked weight of both LT- and HT-dried spaghetti from MACS 1967. On the other hand, there was no significant difference between cooked weight values of LT-dried and HT-dried spaghetti samples. Increase in cooked weight during cooking of LT- and HT-dried spaghetti from PDW 274 is shown in Fig. 56. Significant but low differences were found between cooked weight of LT- and HT-dried spaghetti from PDW 274. A similar significant correlation

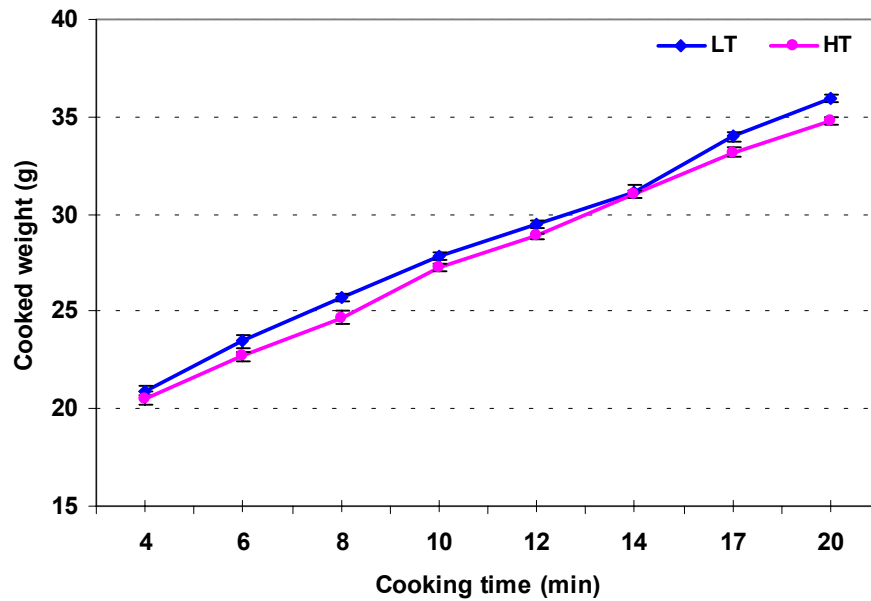


Fig. 55. Cooked weight of spaghetti from good variety MACS 1967 (dried at HT and LT) in different cooking times

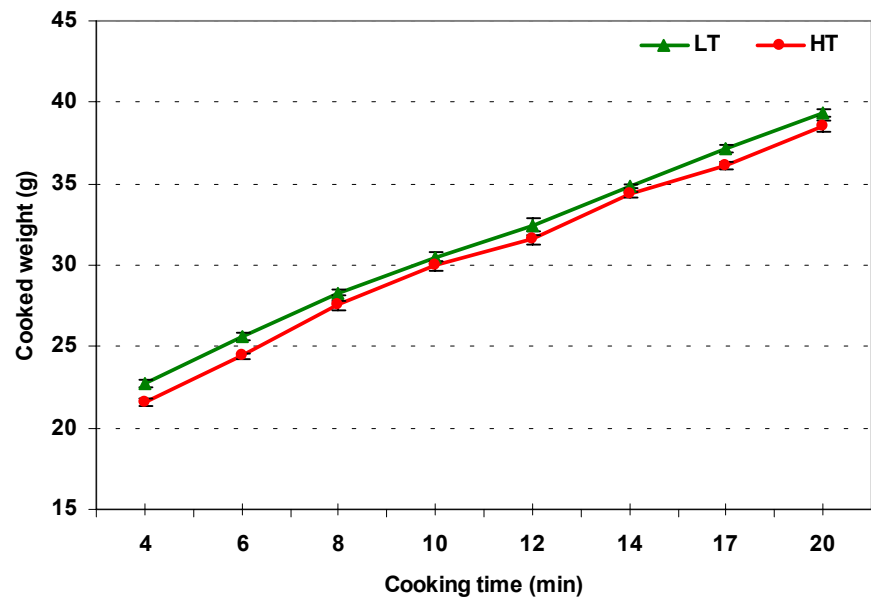


Fig. 56. Cooked weight of spaghetti from poor variety PDW 274 (dried at HT and LT) in different cooking times

( $r=0.98^{**}$ ) was found between cooking time and cooked weight of both LT- and HT-dried spaghetti from PDW 274. However, cooked weight values of both LT- and HT- dried spaghetti from PDW 274 were significantly ( $p<0.05$ ) higher than those of MACS 1967. Higher protein content in semolina from MACS 1967 and denaturation of this protein on the surface of spaghetti strands during cooking could be responsible for reduced water uptake of spaghetti strands leading to a lower cooked weight.

Firmness of spaghetti samples from variety MACS 1967 decreased as cooking time increased (Fig. 57). However, the negative relationship between cooking time and firmness was more linear ( $r=-0.95^{**}$ ) for LT-dried spaghetti compared to HT-dried spaghetti ( $r=-0.91^{**}$ ). Firmness of HT-dried spaghetti was highly affected by cooking times between 6 and 12 min and steadily decreased after 12 min. It is probable that after 12 min of cooking, starch was getting completely gelatinized leading to softening of the product. Presence of higher degree of insoluble proteins and protein-starch interactions in HT-dried spaghetti was probably responsible for maintaining firmness in HT-dried spaghetti during cooking and overcooking.

Firmness of HT-dried spaghetti from PDW 274 was significantly higher than that of LT-dried spaghetti at all cooking times (Fig. 58). However, similar to spaghetti from MACS 1967, decrease in firmness of HT-dried spaghetti was less correlated ( $r=-0.87^{**}$ ) with cooking time than that of LT-dried spaghetti ( $r=-0.90^{**}$ ). The results show a significant improvement in the firmness of spaghetti from poor variety PDW 274 due to HT drying.

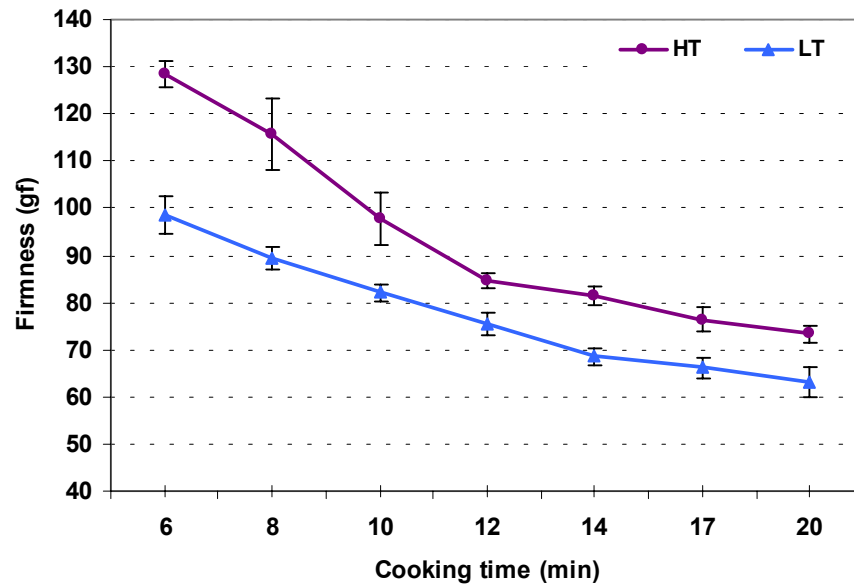


Fig. 57. Firmness of spaghetti from good variety MACS 1967 (dried at HT and LT) in different cooking times

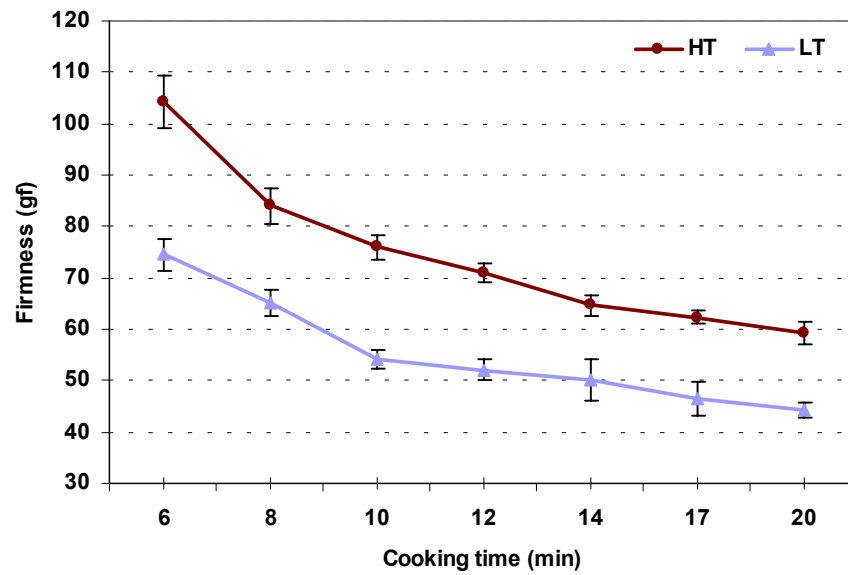
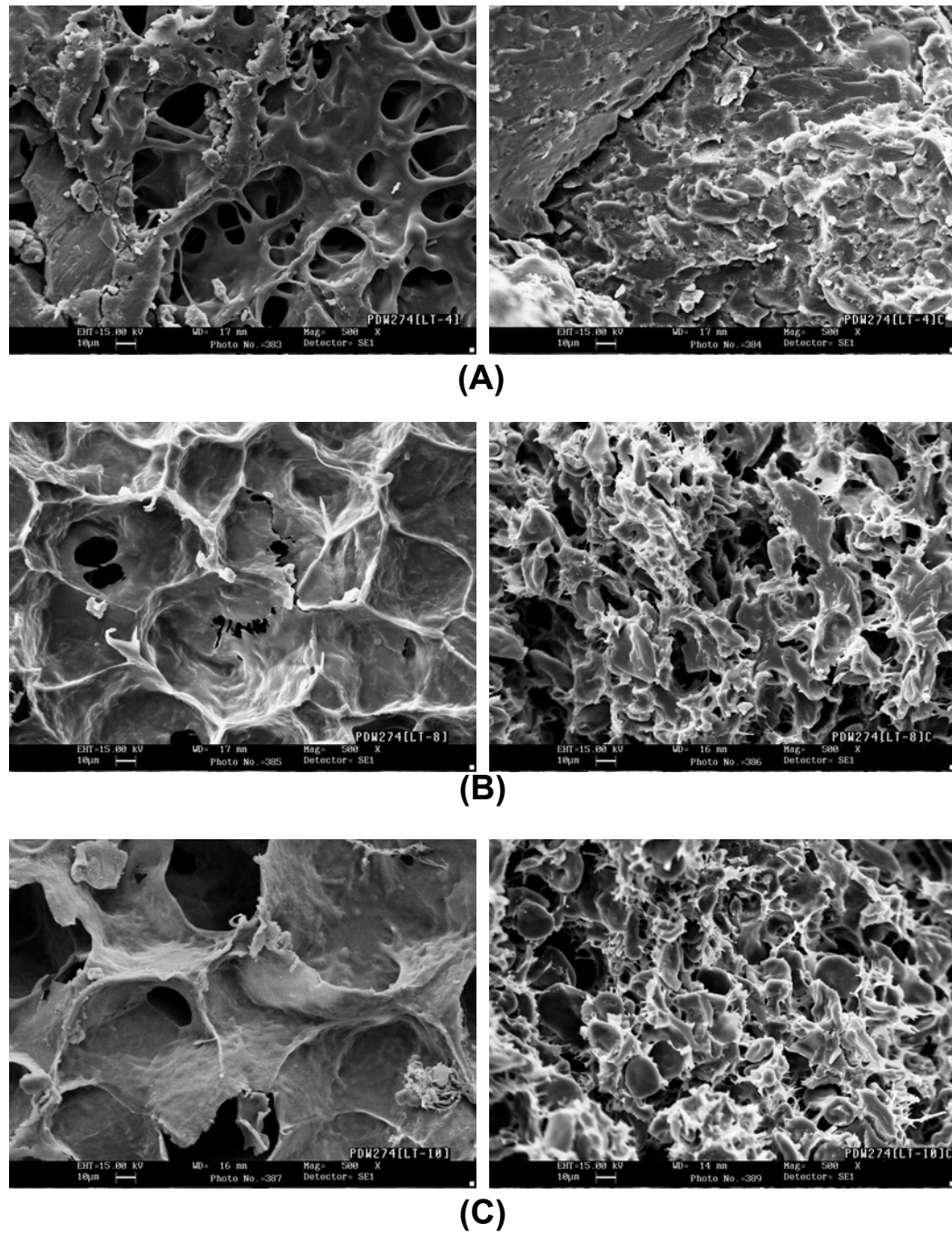


Fig. 58. Firmness of spaghetti from poor variety PDW 274 (dried at HT and LT) in different cooking times

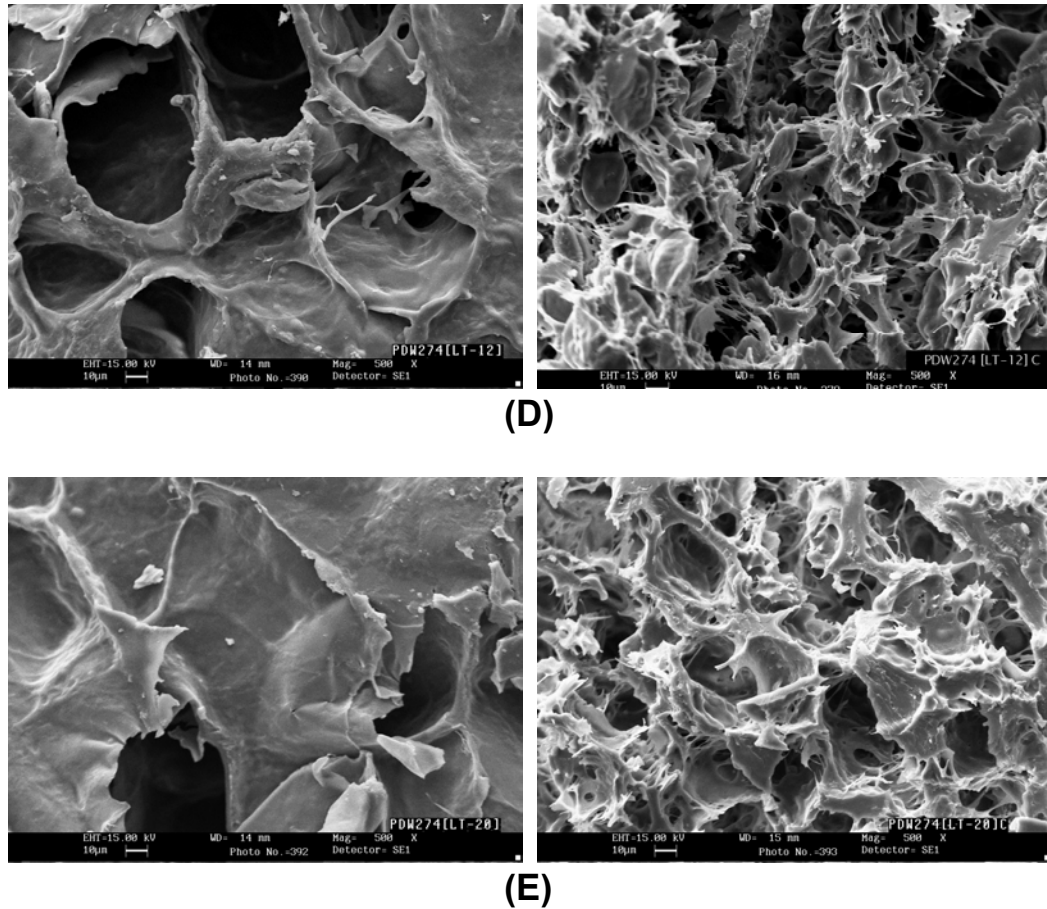
#### **4.3.8. Effect of cooking time on microstructure of spaghetti**

Structural changes of the surface and cross-section (core) of LT and HT-dried spaghetti from poor variety PDW 274 cooked for 4, 8, 10, 12 and 20 min are shown in Fig. 59 and 60, respectively. The surface of the spaghetti samples seem to have undergone a noticeable change and become smoother due to cooking and overcooking. Starch granules seem to have lost their granular shape and appear to be fused together. Some open areas interconnected by fibrils are also visible on the surface of spaghetti cooked for 4 min. This is similar to the structure of Japanese noodles cooked for 6 min as described by Dexter et al (1979). This fibrillar network which in its cross-section appears like a 'honey comb' structure has been also described by other authors (Chabot et al., 1976; Dexter et al., 1978) as a structure comprised of gluten proteins and material leached from starch granules during gelatinization. In other words, it is a protein network that is covered with a thin starch film. As cooking time increased, the network became progressively more open and extensive.

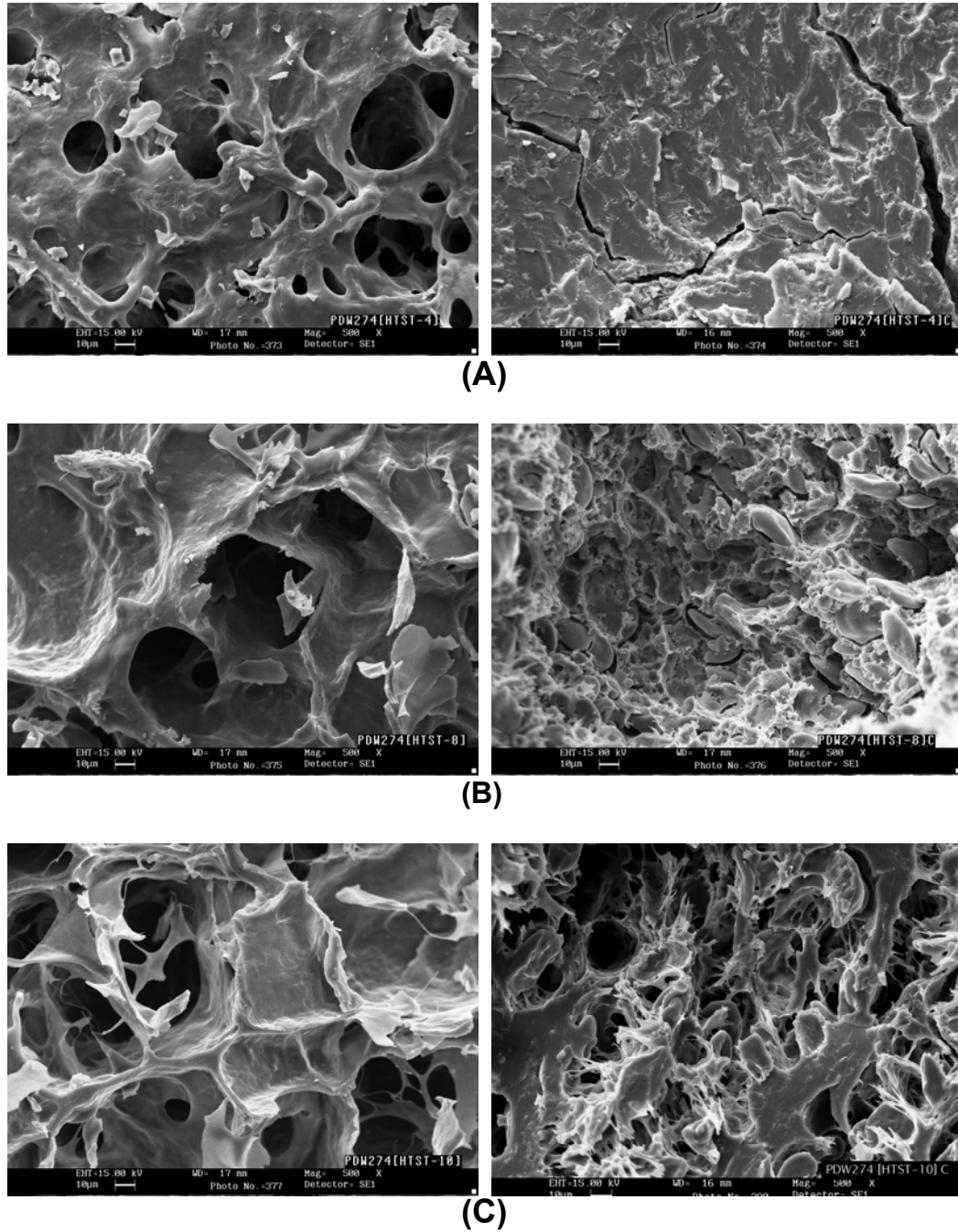
On the other hand, significant differences were observed between the surface structure of LT- and HT-dried spaghetti cooked for different periods of time. Surface of HT-dried spaghetti cooked for 4 min, showed less number of holes and more integrity than that seen in LT-dried spaghetti. The thickness, density and integrity of the surface network of HT-dried spaghetti was more than that observed in the corresponding LT-dried spaghetti, particularly after 12 and 20 min of cooking. This continuous surface, which is probably the result of higher degree of protein coagulation in HT-dried spaghetti, prevents



**Fig. 59.** Scanning electron micrographs of surface (left) and core region (right) of LT-dried spaghetti from poor variety PDW 274 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.

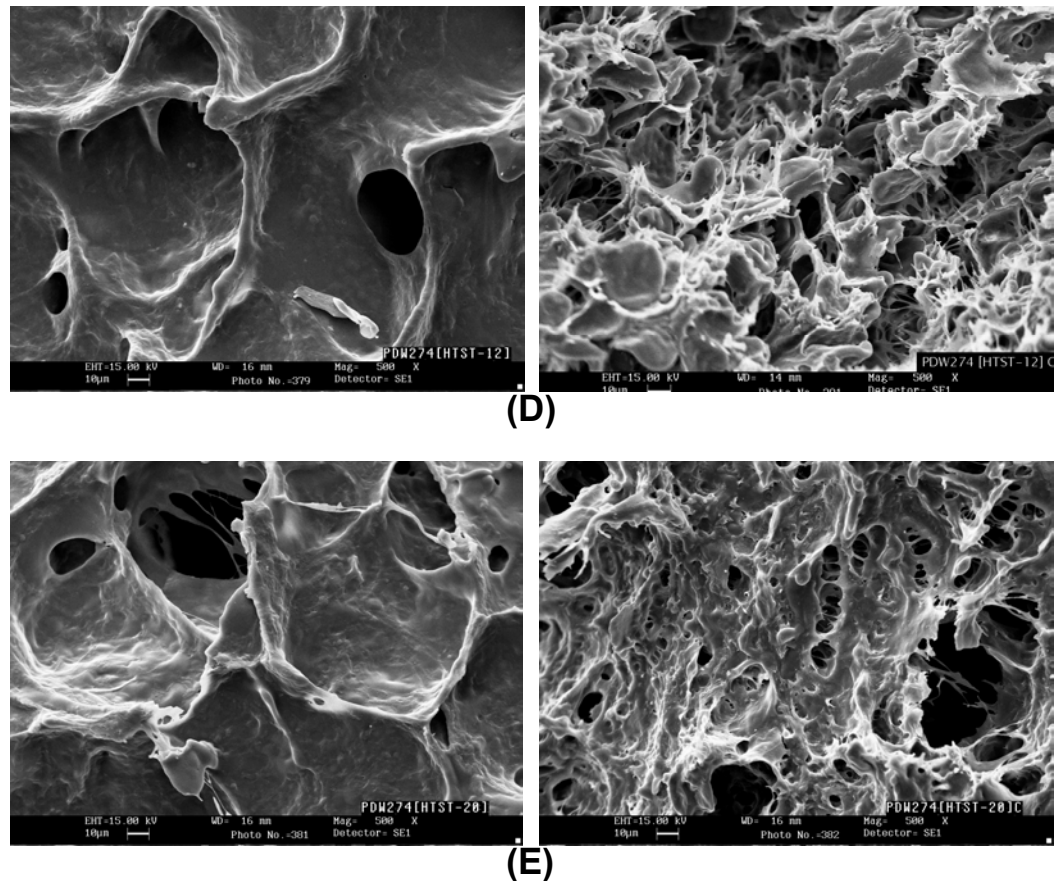


**Fig. 59 (Continued).** Scanning electron micrographs of surface (left) and core region (right) of LT-dried spaghetti from poor variety PDW 274 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.



**Fig. 60.** Scanning electron micrographs of surface (left) and core region (right) of HT-dried spaghetti from poor variety PDW 274 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.





**Fig. 60 (Continued).** Scanning electron micrographs of surface (left) and core region (right) of HT- dried spaghetti from poor variety PDW 274 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.

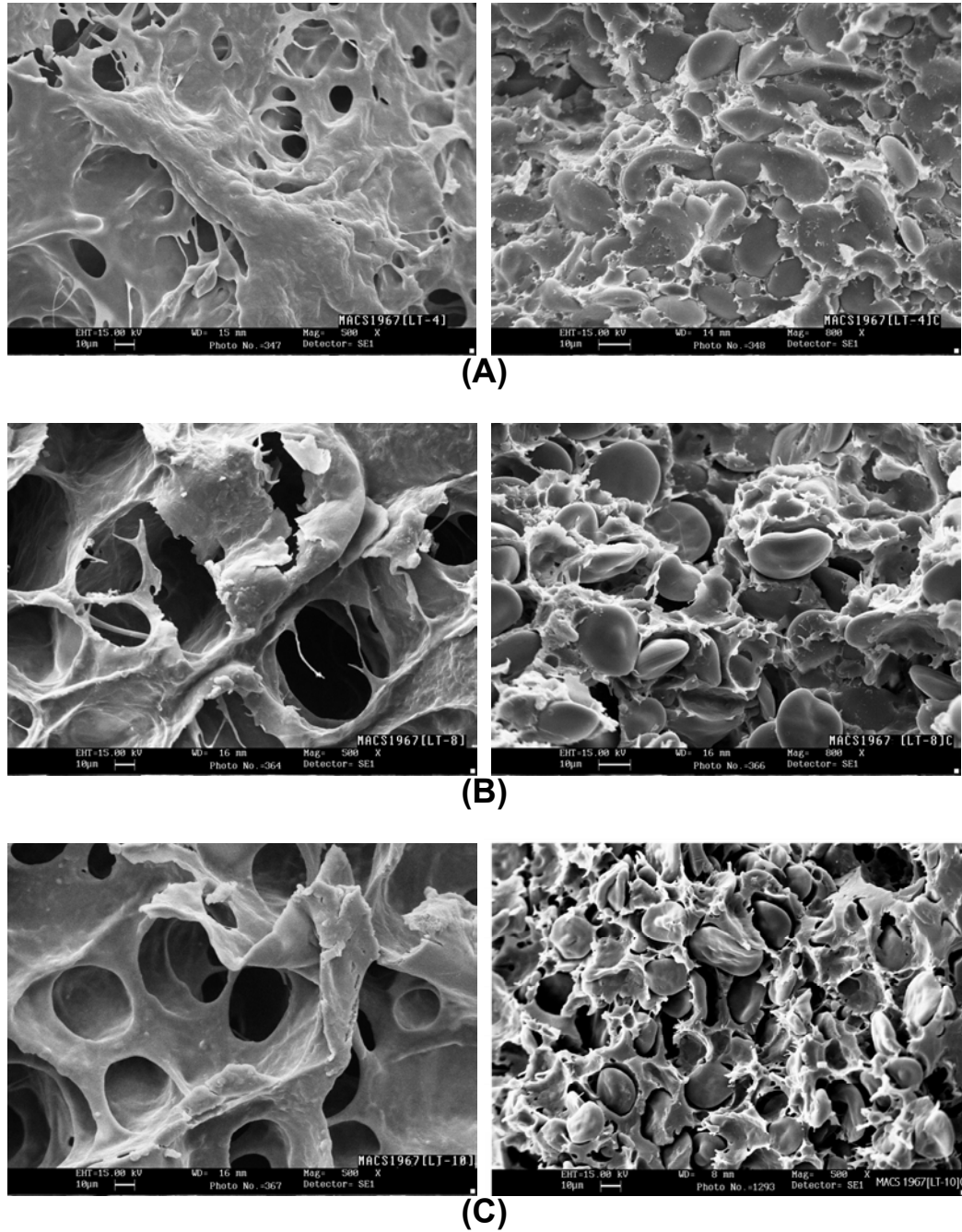
leaching of the starch materials from the interior. As seen earlier, this surface appeared torn, weaker and more fragile in the case of LT-dried spaghetti from PDW 274. The above differences in the microstructure of LT- and HT-dried

spaghetti from PDW 274 might explain the better cooking quality of the latter especially with respect to low cooking loss and low surface stickiness.

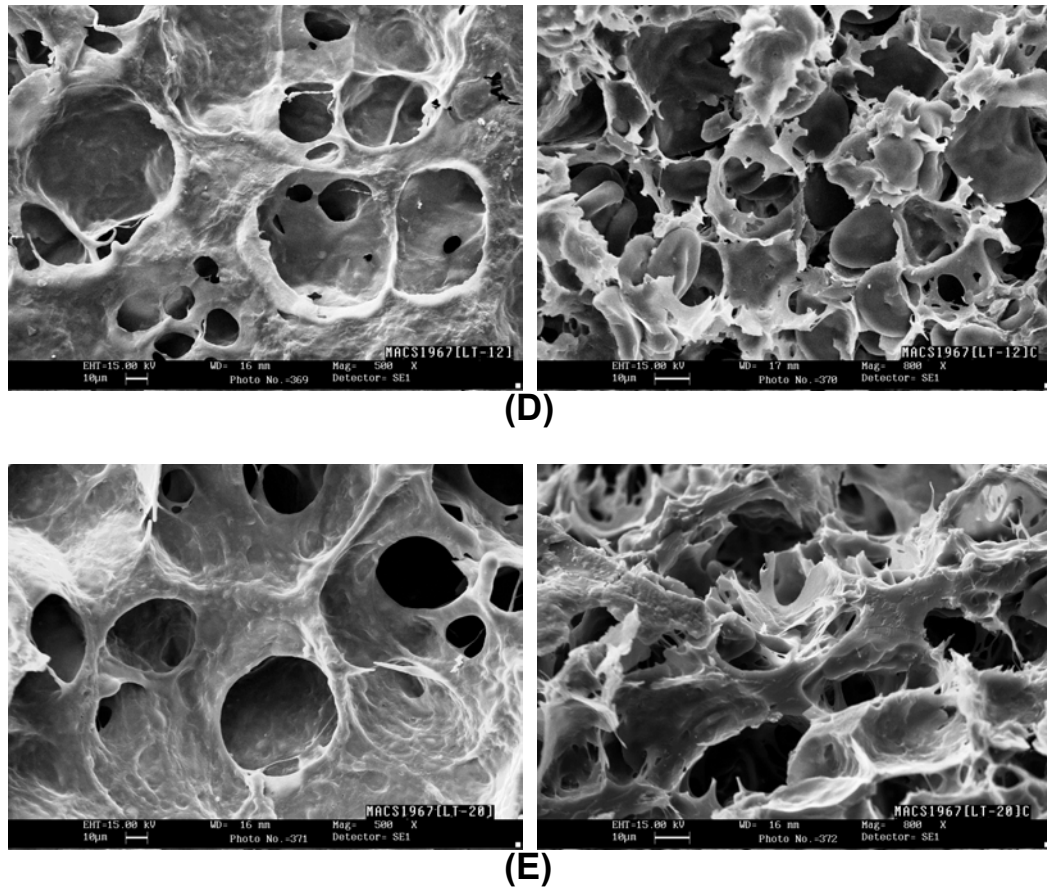
Scanning electron micrographs of internal structure (core region) of cooked LT- and HT-dried spaghetti from poor variety PDW 274 shows a continued change in its structure during cooking from 4 to 20 min. The structure of core region of spaghetti cooked for 4 min where cooking water had not yet penetrated the internal region was similar to that of uncooked spaghetti which composed of starch granules deeply embedded in protein matrix. This structure seemed to be slightly affected in the case of LT-dried spaghetti, whereas in HT-dried spaghetti it was completely intact. Some cracks were visible in the core of HT-dried spaghetti which might be probably due to excessive swelling of starch granules and subsequent expansion of outer layers. Although more number of starch granules were deformed and gelatinized during cooking, some intact starch granules were still visible even after 12 min of cooking, in both LT- and HT-dried spaghetti from PDW 274. However, by the end of 20 min cooking period, almost all the starch granules had got gelatinized hence no intact granule was seen. Significant differences were apparent between cross-section micrographs of LT- and HT-dried spaghetti. For example, after 8 min of cooking a continuous protein matrix surrounding the swollen or intact starch granules was quite visible in the core of HT-dried spaghetti, whereas in the corresponding LT-dried spaghetti most of the starch granules seemed to have lost their shape and were embedded in a discontinuous protein network. Similarly, after 20 min cooking, a continuous structure composed of starch-coated protein network was observed in the core region of HT-dried spaghetti. Dexter et al (1978) pointed out that the

extent (quantity) and strength (quality) of the protein network in the interior part of cooked spaghetti may be important in determining spaghetti cooking quality. In the present study, presence of higher amount of insoluble protein (Table 27), produced as a result of higher degree of protein denaturation in HT-dried spaghetti, might be the reason for high integrity of the protein network in the interior region of its corresponding cooked spaghetti. This might be the reason for the better cooking quality, especially an improvement in firmness of HT-dried spaghetti from PDW 274.

Scanning electron micrographs of surface and cross-section (core) of LT- and HT-dried spaghetti from good variety MACS 1967 cooked for varied time periods are presented in Fig. 61 and 62, respectively. Even though changes in surface structure followed the same pattern as that was discussed for spaghetti from PDW 274, nevertheless, significant differences were apparent between surface of cooked spaghetti samples from MACS 1967 and those of PDW 274. A uniform honeycomb-like starch-coated protein network was highly apparent on the surface of HT- dried spaghetti from MACS 1967 cooked for 4 min. During subsequent cooking and over cooking, this structure was transformed into a more continuous, thick and highly dense network with a much higher degree of integrity. Superior cooking quality of HT-dried spaghetti from MACS 1967 can most probably be related to this surface structure comprising of a continuous protein network.



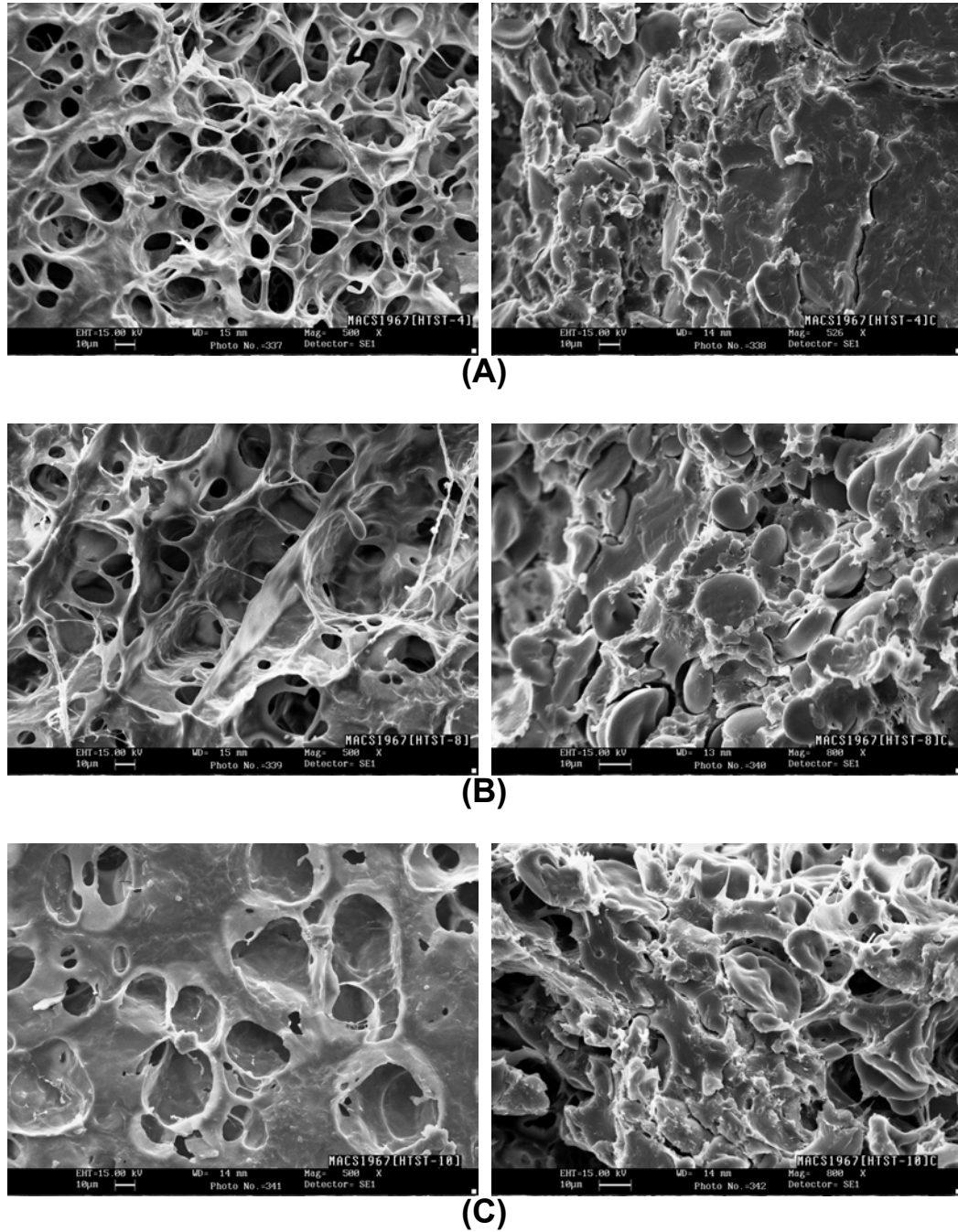
**Fig. 61.** Scanning electron micrographs of surface (left) and core region (right) of LT-dried spaghetti from good variety MACS 1967 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.



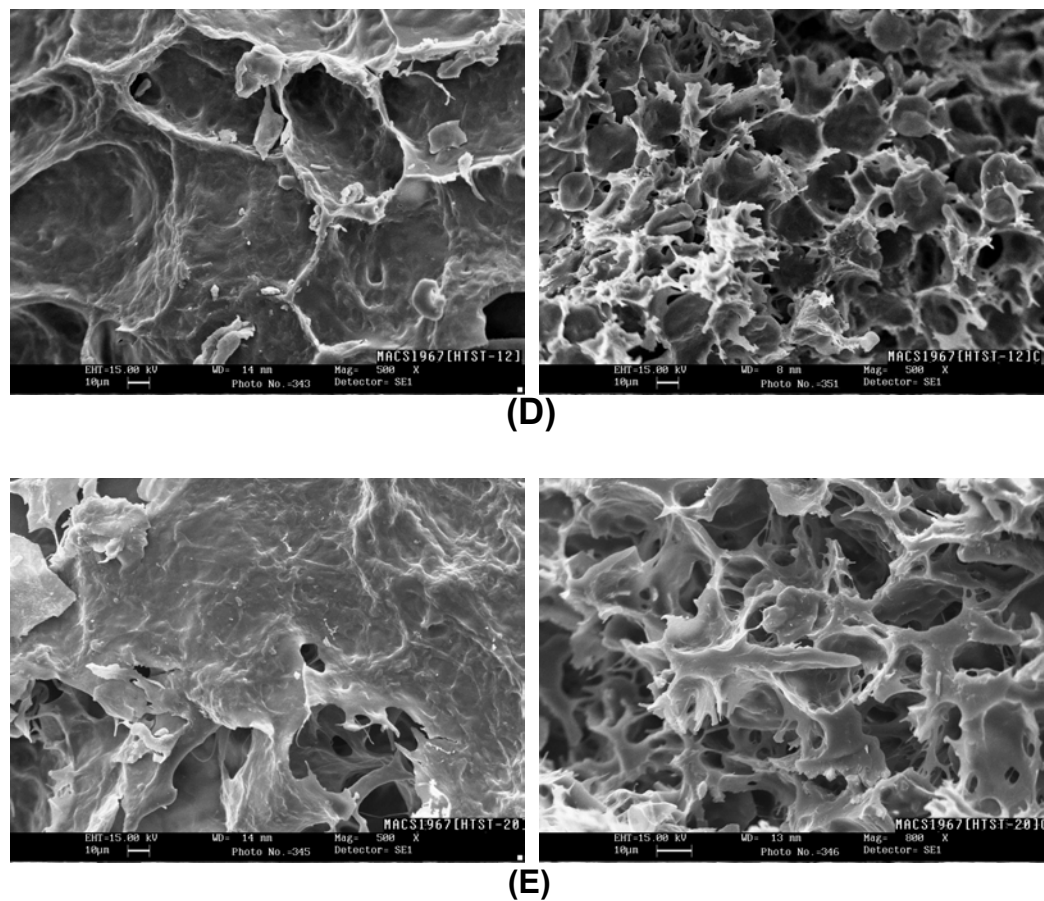
**Fig. 61 (Continued).** Scanning electron micrographs of surface (left) and core region (right) of LT- dried spaghetti from good variety MACS 1967 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.

Similarly, significant differences were observed between core region of LT- and HT-dried spaghetti from MACS 1967 and also between spaghetti from MACS 1967 and PDW 274. Higher protein quantity and quality in variety

MACS 1967 could be the reason for these differences. In the core region of HT-dried spaghetti from MACS 1967, cooked for 10 min, still an area composed of intact starch granules embedded in a continuous protein matrix was visible. These results show that spaghetti from MACS 1967 had the ability to withstand overcooking, at least to some extent, without losing its firm texture. Superior cooking quality (particularly firmness) of HT-dried spaghetti from this variety can be attributed to its internal structure, which showed higher integrity and density than those of other samples.



**Fig. 62.** Scanning electron micrographs of surface (left) and core region (right) of HT-dried spaghetti from good variety MACS 1967 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.

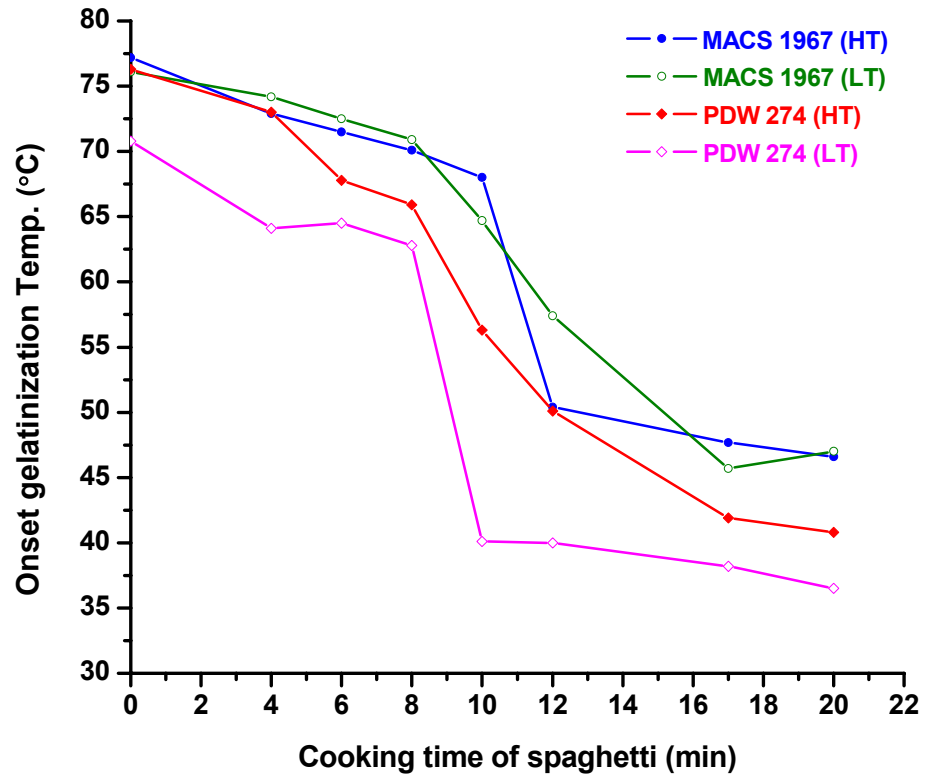


**Fig. 62 (Continued).** Scanning electron micrographs of surface (left) and core region (right) of HT- dried spaghetti from good variety MACS 1967 cooked for 4 (A), 8 (B), 10 (C), 12 (D), and 20 (E) min.

#### 4.3.9. Effect of cooking time on pasting properties of spaghetti



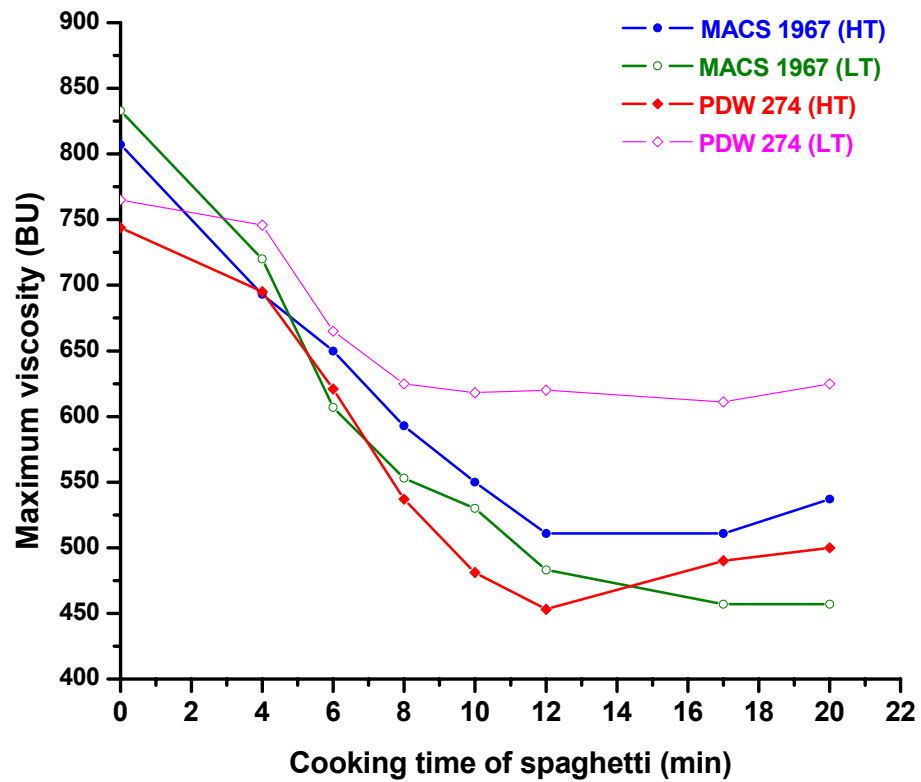
Amylograph studies of the freeze-dried samples of LT- and HT-dried spaghetti from poor variety PDW 274 and good variety MACS 1967 cooked for different times (4-20 min), were carried out to study the changes taken place in these samples during cooking. Fig. 63 shows the onset gelatinization temperature of four spaghetti samples cooked for different periods. Though the onset gelatinization temperatures of uncooked spaghetti samples were significantly higher than those of corresponding semolina, it decreased significantly in the cooked samples. The onset gelatinization temperatures of both LT- and HT-dried spaghetti from MACS 1967 were higher than those of PDW 274 at corresponding cooking times. Decrease in onset gelatinization temperature followed a similar trend for LT- and HT-dried spaghetti from PDW 274 and for LT-dried spaghetti from MACS 1967 cooked up to 8 min. This trend continued for 2 more minutes for HT-dried spaghetti from MACS 1967. This trend can be attributed to increase in the intensity of starch gelatinization as the cooking time of spaghetti progressed. Therefore, freeze-dried sample from spaghetti cooked for a shorter time showed higher gelatinization temperature and vice versa. Although onset gelatinization temperature of different samples was significantly different from each other throughout the cooking process, its decreasing trend in LT-dried spaghetti from MACS 1967 and HT-dried spaghetti from PDW 274 was similar to each other. The lowest onset gelatinization temperature was seen in freeze-dried sample of LT-dried spaghetti from PDW 274 cooked for 10 min. No further change was noted in samples cooked beyond 10 min. Onset gelatinization temperature of 20 min cooked spaghetti was in the significant order of: LT- and HT-dried spaghetti



**Fig. 63.** Onset gelatinization temperature of freeze-dried samples of spaghetti cooked for different time durations

from MACS 1967 > HT-dried spaghetti from PDW 274 > LT-dried spaghetti from PDW 274. On the other hand, onset temperatures of cooked spaghetti from MACS 1967 (HT and LT) was higher than those of PDW 274 (HT and LT). Earlier, Park and Baik (2004a) were able to predict the optimum cooking time of noodles using the changes in amylograph onset temperature of noodles during cooking. Considering that the optimal cooking time is defined as the time in which starch is completely gelatinized in the center of cooked spaghetti and also taking into consideration the electron micrographs of cooked spaghetti, optimal cooking time for LT-dried spaghetti from PDW 274 and HT-dried spaghetti from MACS 1967 can be considered as 10 and 12 min, respectively, because their onset temperature did not change significantly with further cooking up to 20 min.

Fig. 64 shows the changes in maximum viscosity of freeze-dried samples of spaghetti cooked for different time durations. All spaghetti samples except for LT-dried spaghetti from PDW 274 showed a decreasing trend in their peak viscosity starting from 0 min cooking to 12 min cooking. There was no further decrease in peak viscosity of these samples cooked beyond 12 min. The maximum viscosity of freeze-dried samples of LT-dried spaghetti from PDW 274 was slightly different from those of the other three. Maximum viscosity of 4-min cooked spaghetti was slightly lower than that of the raw sample. Even though a decrease in viscosity was observed in samples cooked between 4 and 8 minutes, the viscosity values did not change in samples cooked beyond 8 min. In addition, maximum viscosity of freeze-dried samples cooked beyond 8 min was significantly higher than the other three



**Fig. 64.** Maximum viscosity of freeze-dried samples of spaghetti cooked for different time durations

samples at corresponding cooking times. Although many factors such as protein content, starch characteristics and also interactions between starch and protein due to different drying conditions might influence the viscosity properties, our observation indicated that maximum viscosity of cooked spaghetti rather than onset gelatinization temperature, can serve as a good indicator in determining optimal cooking time of spaghetti. Accordingly, a 12 min cooking might be optimal for HT- and LT-dried spaghetti from MACS 1967 and also HT-dried spaghetti from PDW 274, whereas 8 min might be optimal for cooking of LT-dried spaghetti from PDW 274.

#### **4.3.10. Conclusions**

The present study indicated significant improvement in retention of yellow pigment and in color characteristics of spaghetti from three Indian durum wheats, namely, MACS 1967, DWR 2006 (good varieties), and PDW 274 (poor variety) processed by high temperature drying. Changes were also evident in starch pasting properties of spaghetti samples due to LT and HT drying processes. Microstructure of uncooked spaghetti samples was highly affected by HT drying process. On the other hand, high temperature drying significantly changed the protein profile of spaghetti, as indicated by protein solubility fractionation. Insoluble protein fraction was considerably increased due to HT drying process. Probably all of these changes in protein, starch, and structure of HT-processed spaghetti, individually or in combination, in turn influenced the cooking quality parameters, which were enhanced compared to those of LT-processed spaghetti. Lower cooking loss and stickiness and higher firmness value of HT-dried spaghetti might not only be due to changes

in protein, but also due to changes in starch fraction and also due to starch-protein interactions that would have positively affected the cooking quality of HT-dried spaghetti.

#### 4.4.1. Introduction

Enzymes are interesting alternatives to chemical improvers because they are generally recognized as safe (GRAS) and do not remain active in the food product after cooking and baking (Caballero et al., 2005). Transglutaminase (TG) (protein-glutamine: amine  $\gamma$ -glutamyl-transferase, EC 2.3.2.13) catalyzes an acyl-transfer reaction between an amide group in a protein-bound glutamine and an  $\epsilon$ -amino group in a protein-bound lysine side chain thereby forming covalent cross-links due to  $\epsilon$ -( $\gamma$ -Gln)-Lys bonds, without reducing the nutritional value of the lysine residue (Seguro et al., 1996; Bauer et al., 2003a). Microbial transglutaminase (MTG) which is isolated mostly from *Streptoverticillium* sp., was first introduced in 1989 (Ando et al., 1989; Motoki et al., 1989). It has since been commercialized as a food enzyme preparation by Ajinomoto Co., Inc. This is the only transglutaminase product that is available commercially at present. It has been widely used for protein modification in recent years due to its mass production and also considerably lower cost than mammalian (guinea pig liver) TG. The MTG is stable between pH 5 and 9, which is the pH range for most food processing. MTG is active over a wide range of temperatures with an optimum temperature of 50 °C, and it fully sustains its activity even at 50 °C for 10 min. In contrast with mammalian TG, MTG is characterized by a calcium-independent activity (Motoki and Seguro, 1998; Kuraishi et al., 2001). The results of many studies suggest that MTG, as well as other transglutaminases, has many potential applications in food processing and in other areas. This enzyme has no obvious food safety implications and has been approved for food use in Japan (Gerrard et al., 2000). Due to its unique characteristics, the use of TG as an

ingredient for food processing is increasing not only in Japan but world wide. TG has been affirmed as GRAS by an independent panel of scientific experts (Kuraishi et al., 2001). MTG treatment has been widely used to improve wheat dough and bread quality (Gerrard et al., 1998, 2001; Basman et al., 2002a, Tseng and Lai, 2002; Bauer et al., 2003a,b; Rosell et al., 2003).

Besides the use of TG in bread, positive effects of this enzyme on volume, texture, and intrinsic structure have also been observed in biscuits, puff pastries, cookies and cakes (Ashigawa et al., 1990; Kuraishi et al., 1997; Gerrard et al., 2000; Kuraishi et al., 2001). TG has also been used to produce rice bread, providing a protein network capable of holding the gas produced during proofing, yielding a rice bread with an acceptable specific volume and crumb strength (Gujral and Rosell, 2004). A number of food applications of MTG focus on increasing the functional value of dairy, meat and fish and soybean products (Motoki and Seguro, 1998; Kuraishi et al., 2001).

Though various enzymes have long been used in industrial baking, little has so far been reported about the use of enzymes in the production of pasta (Poutanen, 1997). Utilization of MTG in paste products is limited to the work of Sakamoto et al (1996) and Wu and Corke (2005) on fresh and dried noodles, respectively. However, there is a lack of information regarding the effect of MTG on semolina properties and pasta, especially its effect on spaghetti quality.



Similar to TG and other exogenous enzymes, the use of lipase in the manufacture of noodles and pasta has been limited. Lipase has recently been recognized as a strong dough-conditioning enzyme and has shown excellent effects on bread performance (Si and Lustenberger, 2002). It has been found that the addition of a fungal lipase to flour dough does not adversely affect the rheological properties of the dough measured by farinograph and extensograph (Si, 1996).

Durum wheat lipids, although only a minor constituent, play a significant role in starch pasting properties and spaghetti making quality (Dahle and Muenchow, 1968; Medcalf et al., 1968; Laignelet, 1983; Matsuo et al., 1986). Therefore, any changes in lipids due to lipolytic activities or emulsification could be expected to affect the semolina properties and spaghetti quality.

“Noopazyme” which is a purified lipase produced by submerged fermentation of a genetically modified *Aspergillus oryzae* has been newly introduced by Novozymes company. The application of this fungal lipase has been found to improve the quality of Asian noodles and non-durum pasta (Si and Lustenberger, 2002). However, the effect of this enzyme on the quality of durum wheat pasta, particularly spaghetti, has not yet been reported. The overall performance of microbial lipase, like other enzymes and additives, depends on the procedure, formulation and raw material and presence of other improving ingredients.

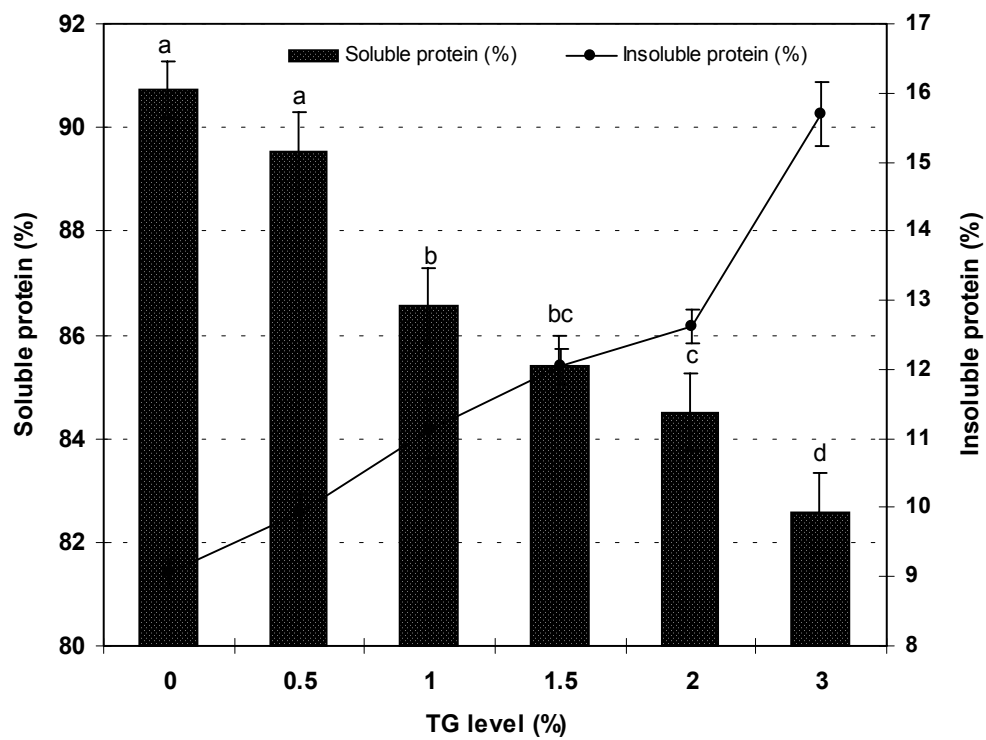
In the present study, effect of microbial TG and microbial lipase on semolina properties and spaghetti quality were investigated. In addition, a commercial soy protein isolate (SPI) alone and in combination with TG (TG + SPI); and also a surfactant namely, distilled glycerol monostearate (DGMS) alone and in combination with lipase (Li + DGMS) were used to study their individual, synergistic or antagonistic effects on rheological properties of semolina and on spaghetti quality. Durum wheat varieties MACS 1967 and PDW 274, which had good and poor spaghetti making properties, respectively, were used in the present study for the production of semolina and spaghetti. Spaghetti samples were processed by high temperature (85 °C) drying method.

#### **4.4.2. Effect of TG treatment on the solubility of protein**

In order to investigate the effect of TG on the solubility of protein in spaghetti dough, semolina from durum variety MACS 1967 was mixed into dough along with different levels of TG (0.5, 1.0, 1.5, 2.0, 3.0%, w/w). The TG levels selected were higher than those reported in literature (maximum 1.5% w/w) for common wheat flour and bread dough (Caballero et al., 2005; Köksel et al., 2001) because the amount of water used for the preparation of pasta dough is almost half of that used for bread dough preparation. Moreover, in contrast to bread dough, pasta dough is prepared in a short period of time (15 min) and the dough development is not completed. A highly denaturing and reducing buffer containing SDS (4%, w/v) and  $\beta$ -mercaptoethanol (5%, v/v) was used to solubilize the protein of freeze-dried dough samples to ensure complete denaturation of proteins and also reduction of disulfide bonds.

Therefore, any changes in protein solubility could be attributed to the cross-linking effect of TG.

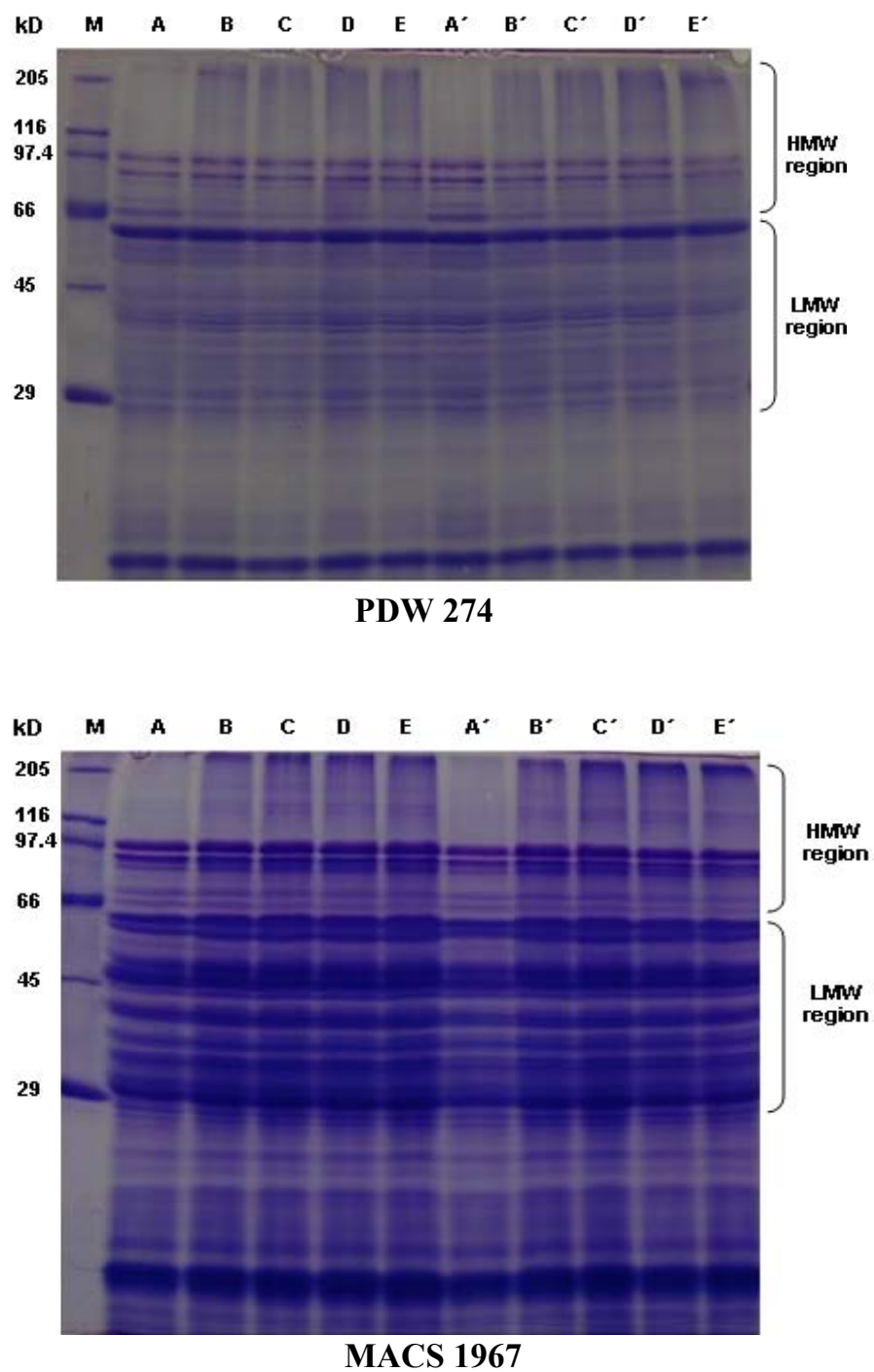
The effect of TG on the solubility of proteins present in spaghetti dough is shown in Fig. 65. Solubility of proteins significantly decreased due to treatment with TG. Concomitantly, the percentage of insoluble protein increased in similar fashion. It can be contemplated that increase in the percentage of insoluble proteins is due to cross-links formed by TG. Earlier, Larré et al (2000) studying the solubility of TG treated common wheat flour, at micro level and using 0.1 M acetic acid, found that the proportion of proteins soluble in acetic acid decreased drastically after TG treatment due to the formation of large insoluble polymers. However, insolubility of these polymers in a buffer containing 5%  $\beta$ -mercaptoethanol that was used in the present study, confirmed the formation of non-disulfide covalent cross-links due to TG treatment. It can be expected that the presence of high concentrations of residue proteins as a result of TG treatment, might have a positive effect on the cooking quality of spaghetti, irrespective of technological, handling and machining properties. This effect could be similar to the positive effect of the presence of high concentrations of insoluble proteins on the cooking quality of spaghetti observed as a result of high temperature drying of spaghetti. It was observed in the present experiments that extrusion of dough containing TG levels higher than 2% (w/w) produced very weak and fragile strands that were found not suitable for further processing. Bauer et al (2003b) explained that TG can be used to modify the functional properties of wheat gluten, but care has to be taken not to use too high a concentration of this enzyme as this may lead to a complete loss of gluten functionality. Therefore, in the present series of studies maximum level of TG used was maintained at 2%.



**Fig. 65.** Effect of TG on protein solubility of mixed spaghetti dough. Data expressed as percentage of total protein of dough (dry basis). Values are mean  $\pm$ SD. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

#### **4.4.3. Effect of TG on SDS-PAGE pattern of spaghetti dough proteins**

SDS-PAGE analysis (under reducing conditions) of total protein of dough from varieties PDW 274 and MACS 1967, before and after extrusion, (Fig. 66) clearly showed that polymerization of protein occurred, and that the degree of polymerization increased with increase in TG concentrations (0.5, 1.0, 1.5, 2.0%, w/w). A progressive decrease in the intensity of the bands corresponding to molecular weight of around 66 kDa was observed when the enzyme concentration increased. This decrease was more evident in variety PDW 274. A slight decrease in intensity was also observed in the molecular weight of around 55 kDa, especially in the extruded dough from PDW 274. On the other hand, new bands corresponding to higher molecular weight molecules (between 116 and 205 kDa) appeared. However, the intensity of these bands decreased in the presence of higher concentration of TG. This may be because of the formation of very high molecular weight polymers some of which were unable to enter the stacking (not shown) and the separating gel. Moreover, marked streaking was observed in the region above molecular weight 97.4 kDa. The degree of streaking was more for extruded dough than for mixed dough. This is confirmed by the results of protein solubility that were discussed earlier. Bauer et al (2003a) working on micro scale treatment of common wheat gluten with TG, found that even after reduction of disulfide bonds, a considerable portion of gluten proteins reached molecular weights up to millions.



**Fig. 66.** SDS-PAGE (in reducing conditions) of PDW 274 and MACS 1967 semolina treated with TG. M: protein markers; A-E: Mixed dough containing 0, 0.5, 1.0, 1.5, 2.0% TG. A'-E': Extruded dough containing 0, 0.5, 1.0, 1.5, 2.0% TG. HMW: high molecular weight; LMW: low molecular weight.

In both the durum varieties, intensity of protein bands with molecular weights lower than 66 kDa did not decrease to a large extent with increase in the concentration of TG. These results indicate that HMW proteins are better substrates than LMW proteins for TG activity. This is in general agreement with the results of Larré et al (2000) and Basman et al (2002b) who worked on aestivum wheat flour. However, Larré et al (2000) showed that both LMW and HMW gluten proteins could be substrates for TG with reactivity of HMW glutenins to be the highest. It should be pointed out that the present study was carried out under conditions that were different from those of Larré et al (2000). In the present study, spaghetti dough was prepared using durum semolina and 35% water with an incubation time of ~15 min, while Larré et al (2000) had used micro scale experiments with refined flour from aestivum wheat with a much longer incubation time. In addition, due to absence of genome 'D' in durum wheat, some of the HMW glutenins that are synthesized by this genome are not present as TG substrates. Therefore, the degree of polymerization in durum wheat dough might be considerably lower than that of aestivum wheat dough as reported in the literature. However, further studies on the degree of reactivity of TG with different HMW-GS need to be investigated.

#### **4.4.4. Effect of soy protein Isolate (SPI) along with TG on semolina properties and spaghetti quality**

It has been reported that soy proteins, such as 11S and 7S globulins act as good substrates for TG reaction (Motoki and Seguro, 1998; Basman et al., 2002b). On the other hand, wheat gluten proteins are rich in glutamine

content (approximately one-third of the total amino acids) but poor in lysine, whereas soy proteins have high contents of lysine (Köksel et al., 2001). A number of studies have shown that TG catalyzes the formation of homologous and heterologous polymers between milk, meat, soybean, and wheat gluten proteins (Motoki and Nio, 1983; Motoki and Seguro, 1998; Babiker, 2000; Basman et al., 2002b). Iwami and Yasumoto (1986) succeeded in introducing lysine into wheat gluten through cross-linking using TG to improve its nutritional value. Then, there is a possibility that TG might create cross-links between wheat gluten proteins (rich in glutamine) and soy proteins (rich in lysine) to improve the functional properties of the wheat flour dough. Therefore, in the present study, a commercial SPI was added at 3% (w/w) level in combination with TG to study their effect on semolina dough properties and on spaghetti quality. Care was taken not to use higher levels of SPI which otherwise might have shown considerable effect independent of the presence of wheat proteins.

#### **4.4.4.1. Effect of TG, SPI and TG+SPI on farinograph characteristics of semolina**

Farinograph experiment of the semolina samples from low protein-poor variety PDW 274, and high protein-good variety MACS 1967, treated with different levels of TG, SPI, and TG + SPI combination, was carried out at constant water absorption of 35% and results summarized in Tables 30 and 31, respectively. Dough development time (DDT) of TG treated samples was significantly ( $p < 0.05$ ) higher than that of control samples in both varieties. However, the effect of TG on DDT was more evident in the case of low protein



**Table 30.** Effects of TG, SPI and TG + SPI on farinograph characteristics of semolina from poor variety PDW 274

	Dough development time (min)	Maximum consistency (FU)	Tolerance index (FU)
<b>Control</b>	6.1 <sup>g</sup>	335.0 <sup>b</sup>	8.0 <sup>cde</sup>
<b><u>TG (%)</u></b>			
0.5	9.0 <sup>d</sup>	325.0 <sup>b</sup>	7.5 <sup>de</sup>
1.0	11.3 <sup>b</sup>	297.5 <sup>c</sup>	10.6 <sup>cd</sup>
1.5	13.3 <sup>a</sup>	249.0 <sup>e</sup>	23.5 <sup>ab</sup>
2.0	9.7 <sup>c</sup>	220.5 <sup>f</sup>	25.0 <sup>a</sup>
<b><u>SPI (%)</u></b>			
3.0	6.8 <sup>f</sup>	370.0 <sup>a</sup>	11.7 <sup>c</sup>
<b><u>TG + SPI (%)</u></b>			
1 + 3	8.2 <sup>e</sup>	336.0 <sup>b</sup>	5.5 <sup>e</sup>
2 + 3	9.8 <sup>c</sup>	282.0 <sup>d</sup>	20.3 <sup>b</sup>

Data are expressed as mean of two determinations. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ).

**Table 31.** Effects of TG, SPI and TG + SPI on farinograph characteristics of semolina from good variety MACS 1967

	Dough development time (min)	Maximum consistency (FU)	Tolerance index (FU)
<b>Control</b>	3.5 <sup>c</sup>	375.0 <sup>c</sup>	15.5 <sup>b</sup>
<b><u>TG (%)</u></b>			
0.5	4.7 <sup>b</sup>	377.5 <sup>c</sup>	8.1 <sup>c</sup>
1.0	5.2 <sup>b</sup>	377.0 <sup>c</sup>	6.4 <sup>c</sup>
1.5	7.6 <sup>a</sup>	371.0 <sup>c</sup>	8.1 <sup>c</sup>
2.0	7.6 <sup>a</sup>	358.0 <sup>d</sup>	31.5 <sup>a</sup>
<b><u>SPI (%)</u></b>			
3.0	3.9 <sup>c</sup>	420.0 <sup>a</sup>	5.2 <sup>c</sup>
<b><u>TG + SPI (%)</u></b>			
1 + 3	4.7 <sup>b</sup>	405.5 <sup>b</sup>	4.2 <sup>c</sup>
2 + 3	4.9 <sup>b</sup>	368.5 <sup>cd</sup>	18.8 <sup>b</sup>

Data are expressed as mean of two determinations. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ).

variety PDW 274. Even though TG level of 2% slightly decreased the DDT of this variety, it was still significantly higher than that of control. DDT of MACS 1967 increased with addition of TG up to 1.5% level, and at 2% no further increase was observed. Addition of 3% SPI did not considerably change the DDT values in both varieties. Dough samples from PDW 274 and MACS 1967 behaved differently in terms of DDT when treated with TG + SPI combinations. Both combinations (1+3 and 2+3) modified the DDT of MACS 1967 to a level lower than those containing their corresponding level of TG alone, but still higher than that of control. In variety PDW 274, combination of 2% TG and 3% SPI could not change the DDT value as a drastic decrease had already taken place due to treatment with 2% TG. Combination of 1+3% (TG+SPI) decreased the DDT of PDW 274 to a significantly lower value than those containing 0.5% and 1% of TG alone. Earlier, Basman et al (2002a) studying on the effect of TG on rheological properties of a low protein soft wheat and a high protein hard wheat flour indicated that DDT value first increased with increasing level of the enzyme (0.25 or 0.5%), but decreased at higher TG addition levels (up to 1.5%). However, the amount of water in their experiments (~56% to ~ 63%) was quite higher than that used in the present study for semolina (35%). In the presence of a better dough formation due to finer flour particle size and higher water absorption, a more intensive TG activity could be expected in their study.

Farinograms of semolina from varieties PDW 274 and MACS 1967, treated with different levels of TG, showed different response in terms of maximum consistency (MC). While increasing levels of TG was associated

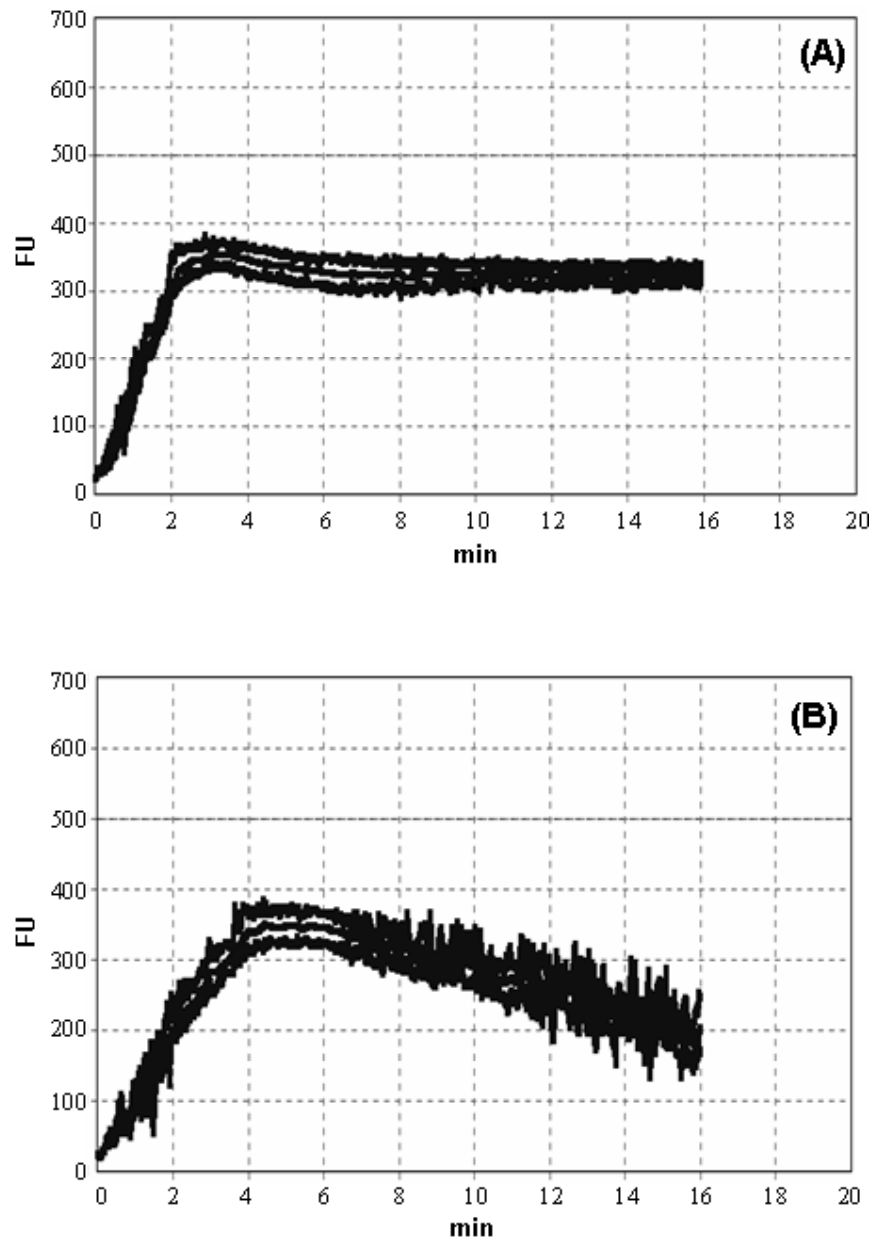
with decrease in MC of dough from PDW 274, MC of dough from MACS 1967 was not affected significantly ( $P < 0.05$ ) up to 1.5% of TG addition, but significantly decreased due to treatment with 2% of TG. Addition of 3% SPI alone significantly increased the MC of dough from both varieties to the highest level compared to rest of the other samples. The farinograms of both varieties also showed that SPI in combination with TG significantly increased the MC of the dough compared to those containing corresponding levels of TG alone. In high protein semolina (MACS 1967), combination of 1+3% (TG+SPI) increased the MC to 405.5 F.U., which was significantly higher than that of control (375 F.U). The improving effect of SPI confirms findings of Bauer et al (2003b) who pointed out that, as gluten protein is a good substrate for TG, the enzyme might be used to incorporate other proteins such as soybean proteins covalently into gluten with minimal loss of its functionality. Furthermore, gluten-free proteins might be cross-linked by TG to create functional properties that are comparable to gluten.

Results of the present study on the effect of TG on MC were found to be similar to those of Gerrard et al (1998) and Bauer et al (2003b). On the other hand, Basman et al (2002a) showed that addition of increasing levels of TG was associated with decrease in water absorption. However, Gerrard et al (1998) reported that addition of TG to dough lowered the height of the mixing curve, indicating that less water should be added. But these authors showed that immediately after mixing, the TG treated doughs felt too tight and their corresponding breads appeared to have had insufficient water addition. They found a 6% extra water addition ideal for TG treated doughs and

corresponding breads. Although the reasons for the increased water absorption of the TG treated dough is not clear, altering the structure of the gluten by cross-linking may lead to an increased capacity to hold water (Gerrard et al., 1998). Another alternative explanation might be the results of side activity of TG so that, TG will hydrolyze glutamine residues to glutamic acid residues in protein, resulting in the increase of hydrophilicity of the gluten and higher affinity for water (Alexandre et al., 1993). Based on the above studies, it can be reasoned that decrease in dough consistency observed with addition of TG in the present experiments could be due to changes taking place in gluten proteins, as the amount of water added in the farinograph studies was kept constant at 35%. Subjective observations made during farinograph mixing also indicated that dough from both cultivars treated with higher levels of TG (1.5% and more) developed a dry appearance after few minutes of mixing resulting in a beady granular structure that was weak and highly non-cohesive in nature. Higher values for DDT and delay in dough formation of TG treated samples suggest that water added during mixing was not sufficient to form a cohesive dough due to structural changes taken place in gluten proteins. Though farinograph characteristics of semolina dough with a constant water absorption of 35% might not be comparable with farinograph properties of flour with water absorption of around 63%, our observations are in general agreement with the findings of Gerrard et al (1998) that TG treated doughs require higher levels of water addition.

Mixing tolerance index (MTI) value of dough from low protein variety PDW 274 significantly increased with addition of 1.5 and 2% of TG. However, MTI

of dough from high protein variety MACS 1967 did not change up to 1.5% of TG addition but significantly increased with 2% TG addition. Combination of TG and SPI only at the level of 1+3% could improve (decrease) the MTI of the dough from both varieties. On the other hand, combination of 2+3% (TG+SPI) increased the MTI of dough from both varieties indicating weakening of the dough. However, farinograms of dough from both varieties, treated with 2+3% combination, showed that there was no significant difference between shape of these farinograms and those from corresponding 2% TG treated doughs, after 15 min of mixing. The MTI that is measured 4 min after peak viscosity, as is normally done for semolina, was not able to show this difference properly. In other words, it can be contemplated that the gluten protein had lost its functionality due to high degree of cross-linking by addition of 2% TG so that its tolerance to mixing could not be improved even when added in combination with 3% SPI. This phenomenon was more evident in low protein semolina (PDW 274). A representative farinogram (Fig. 67) of TG+SPI (2+3%) treated dough from MACS 1967 shows the effects of high TG level on the shape of the graph in comparison with farinogram of control. In addition to decrease in MC and a drastic fall in the consistency after few minutes of mixing, fluctuations in the farinograph band were observed, which was due to loss of cohesiveness and homogeneity in the dough. Bauer et al (2003b) reported that due to the formation of cross-linked proteins in the dough, TG affects the structure of the gluten network and therefore its viscoelastic properties. However, these authors pointed out that high level of TG caused excessive cross-linking of the gluten proteins leading to the mechanical damage of the gluten network and loss of elasticity. Kuraishi et al (2001) also



**Fig. 67.** Representative farinograms of semolina from high protein variety MACS 1967 without (A) and with addition of 2%TG + 3%SPI (B).

reported that too high a concentration of TG makes the protein network brittle and leads to a loss of structure. However, it should be pointed out that unlike the bread and bakery products, a completely developed dough is not generally used for pasta production. Moreover, the functional properties of gluten have less importance for the production of pasta. Therefore, data from farinograph properties of TG treated dough might not be directly correlated with pasta making quality of treated dough. The actual effect of different levels of TG in relation to spaghetti quality, can however be evaluated by studying their influence on spaghetti cooking quality.

#### **4.4.4.2. Effect of TG, SPI and TG+SPI on pasting properties of semolina**

Pasting properties of ground semolina treated with different levels of TG, 3% (w/w) SPI, and TG+SPI combinations (1% + 3% and 2% + 3%) was carried out using a micro visco-amylograph to study the effects of protein cross-linking, if any, on amylograph pasting properties. Results of this study are shown in Tables 32 and 33. Different levels of TG, 3% SPI and TG+SPI combinations did not affect the onset gelatinization temperature of semolina from low protein variety PDW 274, while that of high protein variety MACS 1967 increased slightly in presence of 3% SPI and 2%+3% combination of TG and SPI. Increasing levels of TG significantly increased the peak viscosities of semolina from both PDW 274 and MACS 1967. Addition of 3% SPI also significantly increased the peak viscosity of semolina from both varieties. Interestingly, both combinations of TG and SPI increased the peak viscosity in both varieties to the highest levels. The highest peak viscosity in both varieties was observed in semolina treated with 2%TG+3%SPI. The



**Table 32.** Effects of TG, SPI and TG + SPI on pasting properties of semolina from poor variety PDW 274

	Onset gelatinization temp. (°C)	Peak viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)	Final viscosity (BU)
<b>Control</b>	64.1 <sup>a</sup>	748 <sup>f</sup>	90.0 <sup>e</sup>	530.0 <sup>ab</sup>	1267 <sup>f</sup>
<b><u>TG (%)</u></b>					
0.5	63.6 <sup>a</sup>	816 <sup>d</sup>	112 <sup>d</sup>	500.0 <sup>e</sup>	1282 <sup>e</sup>
1.0	64.1 <sup>a</sup>	811 <sup>de</sup>	50.0 <sup>f</sup>	481.5 <sup>f</sup>	1275 <sup>ef</sup>
1.5	63.9 <sup>a</sup>	828 <sup>bc</sup>	97.5 <sup>e</sup>	512.0 <sup>cd</sup>	1306 <sup>d</sup>
2.0	64.1 <sup>a</sup>	818 <sup>cd</sup>	60.0 <sup>f</sup>	513.0 <sup>cd</sup>	1304 <sup>d</sup>
<b><u>SPI (%)</u></b>					
3.0	64.3 <sup>a</sup>	804 <sup>e</sup>	202.5 <sup>b</sup>	506.0 <sup>de</sup>	1404 <sup>c</sup>
<b><u>TG + SPI (%)</u></b>					
1 + 3	64.0 <sup>a</sup>	835 <sup>ab</sup>	187.5 <sup>c</sup>	537.0 <sup>a</sup>	1448 <sup>b</sup>
2 + 3	64.4 <sup>a</sup>	845 <sup>a</sup>	240.0 <sup>a</sup>	519.5 <sup>bc</sup>	1477 <sup>a</sup>

Data are expressed as mean of two determinations. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ).

**Table 33.** Effects of TG, SPI and TG + SPI on pasting properties of semolina from good variety MACS 1967

	Onset gelatinization temp. (°C)	Peak viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)	Final viscosity (BU)
<b>Control</b>	66.3 <sup>cd</sup>	830 <sup>e</sup>	115.0 <sup>c</sup>	522 <sup>b</sup>	1240 <sup>e</sup>
<b><u>TG (%)</u></b>					
0.5	66.6 <sup>bcd</sup>	844 <sup>d</sup>	117.5 <sup>c</sup>	512 <sup>b</sup>	1235 <sup>e</sup>
1.0	66.4 <sup>cd</sup>	856 <sup>cd</sup>	123.5 <sup>bc</sup>	514 <sup>b</sup>	1238 <sup>e</sup>
1.5	66.3 <sup>cd</sup>	863 <sup>c</sup>	115.0 <sup>c</sup>	520 <sup>b</sup>	1265 <sup>d</sup>
2.0	66.2 <sup>d</sup>	880 <sup>b</sup>	121.0 <sup>c</sup>	512 <sup>b</sup>	1272 <sup>d</sup>
<b><u>SPI (%)</u></b>					
3.0	67.1 <sup>a</sup>	857 <sup>cd</sup>	134.0 <sup>b</sup>	600 <sup>a</sup>	1346 <sup>c</sup>
<b><u>TG + SPI (%)</u></b>					
1 + 3	66.7 <sup>abc</sup>	888 <sup>b</sup>	151.0 <sup>a</sup>	584 <sup>a</sup>	1364 <sup>b</sup>
2 + 3	67.0 <sup>ab</sup>	903 <sup>a</sup>	156.0 <sup>a</sup>	596 <sup>a</sup>	1469 <sup>a</sup>

Data are expressed as mean of two determinations. Means in the same column followed by different letters are significantly different ( $p < 0.05$ ).

pasting properties of wheat flour recorded in an amylograph are generally attributed to starch components. However, positive correlations have also been found between amylograph peak viscosity and flour protein content (Moss, 1967, 1980; Moss and Miskelly, 1984). Morris et al (1997) clearly showed the significant effects of different flour fractions on peak viscosity. Based on their work, Morris et al (1997) suggested that the indirect role of gluten in pasting viscosity was probably related to altered water relations, i.e., a sequestration of water of the system such that the effective starch concentration was increased. Momirović-Čuljat and Balint (1976) conducting amylograph studies on papain-digested sample reported that the pasting properties of such a sample were different from that of the control sample. They concluded that any disturbance in the equilibrium of starch-protein in the complex system could affect the peak viscosity. Both of these hypotheses can be correlated to the effect of TG on increasing the peak viscosity, because there is a high possibility that the formation of new cross-links in semolina gluten by TG or TG+SPI could disturb the starch-protein equilibrium. Secondly, the higher affinity of cross-links for water can increase the starch concentration in the slurry resulting in higher peak viscosity.

To ensure that increase in peak viscosity of TG treated samples was not due to maltodextrin which is used as a filler of commercial TG preparation, a commercial maltodextrin was added (2%, w/w) to semolina from MACS 1967 and micro visco-amylograph test was carried out. There was no significant change in peak viscosity compared to control (result not shown).

No certain trend in the breakdown viscosity of semolina from low protein variety PDW 274 was observed with the addition of various levels of TG. Only at 0.5% addition level there was a significant increase in the breakdown viscosity. On the contrary, breakdown viscosity of semolina from the high protein variety MACS 1967, did not change when treated with different levels of TG. Treatment with 3% SPI and both combinations of TG+SPI significantly increased the breakdown viscosity in both the varieties. Lim and Narsimhan (2006) using different soy proteins (5%) mixed with different starches, observed higher pasting temperature, peak viscosity and shear thinning (breakdown) in the soy protein/starch pastes. These effects can be due to some synergistic interaction between soy protein and starch, increase in the concentration of solid contents resulting from the addition of soy proteins, and/or through self-aggregation of soy globulins (Chronakis et al., 1995; Tolstoguzov, 2003; Lim and Narsimhan, 2006). Earlier Wu and Corke (2005) studying the pasting properties of TG treated common wheat flour, using rapid visco-analyzer (RVA), observed an increase in breakdown viscosity with TG levels of 0.5-2%. Assuming that higher breakdown is due to more solubilization of starch during shearing (Lim and Narsimhan, 2006), it can be contemplated that high interactions (cross-links) within gluten proteins and also between gluten and SPI due to TG treatment result in more compactness of protein and less interaction of protein and starch in the dilute system of amylography which results in higher solubilization of starch. Another alternative explanation might be according to the water competition hypothesis of Morris et al (1997), which results in higher concentration of starches thereby higher rupture of starch due to heating. Based on these two

hypothesis, presence of higher solubilized starch and higher concentration of starch in the system might have resulted in higher final viscosity due to the enhanced re-association process of amylose molecules in the treated semolina from both varieties (Tables 32 and 33). Setback viscosities of SPI and TG+SPI (both combinations) treated semolina from high protein variety MACS 1967 were significantly higher than that of control. Higher setback in the presence of soy proteins can be due to increased formation of not only thermally reversible hydrogen bonds but also thermally irreversible hydrophobic and/or covalent bonds because of the heating / holding procedure (Luck et al., 2002; Tolstoguzov, 2003; Lim and Narsimhan, 2006). On the other hand, higher protein content of MACS 1967 along with cross-linking effect of TG would have increased the degree of intensity of above-mentioned phenomena.

#### **4.4.4.3. Effect of TG, SPI and TG+SPI on color characteristic of dry spaghetti**

The importance of color, particularly yellow color of spaghetti on its overall quality and acceptance has already been discussed in the previous chapters. In the present study, color characteristics of dry spaghetti samples measured in Hunter color system and expressed as 'L' (brightness), 'a' (greenness-redness), and 'b' (yellowness) are shown in Table 34 (PDW 274) and Table 35 (MACS 1967). Brightness of spaghetti samples from both varieties significantly ( $p < 0.05$ ) decreased due to TG treatment. 'L' value of spaghetti from PDW 274 continued to decrease with each increase in the level of TG added. At 2% level of addition, 'L' value decreased from 55.58 to

**Table 34.** Effects of TG, SPI and TG + SPI on color characteristics\* of dry spaghetti from poor variety PDW 274

	<b>L</b>	<b>a</b>	<b>b</b>
<b>Control</b>	55.58 ± 0.18 <sup>a</sup>	-0.59 ± 0.08 <sup>c</sup>	22.64 ± 0.19 <sup>a</sup>
<b><u>TG (%)</u></b>			
<b>0.5</b>	53.24 ± 0.32 <sup>b</sup>	-1.25 ± 0.16 <sup>c</sup>	20.55 ± 0.20 <sup>c</sup>
<b>1.0</b>	53.21 ± 0.30 <sup>b</sup>	-1.50 ± 0.14 <sup>f</sup>	20.28 ± 0.32 <sup>c</sup>
<b>1.5</b>	53.24 ± 0.22 <sup>b</sup>	-1.10 ± 0.10 <sup>de</sup>	20.45 ± 0.17 <sup>c</sup>
<b>2.0</b>	52.53 ± 0.21 <sup>c</sup>	-0.96 ± 0.07 <sup>d</sup>	20.64 ± 0.25 <sup>c</sup>
<b><u>SPI (%)</u></b>			
<b>3.0</b>	53.29 ± 0.35 <sup>b</sup>	0.18 ± 0.05 <sup>b</sup>	21.45 ± 0.31 <sup>b</sup>
<b><u>TG + SPI (%)</u></b>			
<b>1 + 3</b>	51.22 ± 0.38 <sup>d</sup>	0.23 ± 0.07 <sup>b</sup>	21.65 ± 0.28 <sup>b</sup>
<b>2 + 3</b>	52.35 ± 0.21 <sup>c</sup>	0.67 ± 0.11 <sup>a</sup>	21.88 ± 0.30 <sup>b</sup>

Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* L: brightness; a: redness-greenness; b: yellowness.

**Table 35.** Effects of TG, SPI and TG + SPI on color characteristics\* of dry spaghetti from good variety MACS 1967

	<b>L</b>	<b>a</b>	<b>b</b>
<b>Control</b>	54.98 ± 0.34 <sup>a</sup>	-0.53 ± 0.07 <sup>c</sup>	21.81 ± 0.25 <sup>a</sup>
<b><u>TG (%)</u></b>			
<b>0.5</b>	52.16 ± 0.21 <sup>d</sup>	-1.11 ± 0.09 <sup>d</sup>	19.28 ± 0.20 <sup>d</sup>
<b>1.0</b>	52.35 ± 0.20 <sup>cd</sup>	-1.17 ± 0.14 <sup>d</sup>	19.11 ± 0.18 <sup>d</sup>
<b>1.5</b>	52.24 ± 0.32 <sup>d</sup>	-1.10 ± 0.11 <sup>d</sup>	19.32 ± 0.31 <sup>d</sup>
<b>2.0</b>	52.85 ± 0.18 <sup>bc</sup>	-1.02 ± 0.08 <sup>d</sup>	19.89 ± 0.12 <sup>c</sup>
<b><u>SPI (%)</u></b>			
<b>3.0</b>	53.08 ± 0.27 <sup>b</sup>	-0.17 ± 0.03 <sup>b</sup>	20.56 ± 0.35 <sup>b</sup>
<b><u>TG + SPI (%)</u></b>			
<b>1 + 3</b>	52.36 ± 0.34 <sup>cd</sup>	-0.20 ± 0.03 <sup>b</sup>	20.14 ± 0.23 <sup>bc</sup>
<b>2 + 3</b>	51.39 ± 0.22 <sup>e</sup>	0.07 ± 0.08 <sup>a</sup>	20.30 ± 0.32 <sup>bc</sup>

Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* L: brightness; a: redness-greenness; b: yellowness.

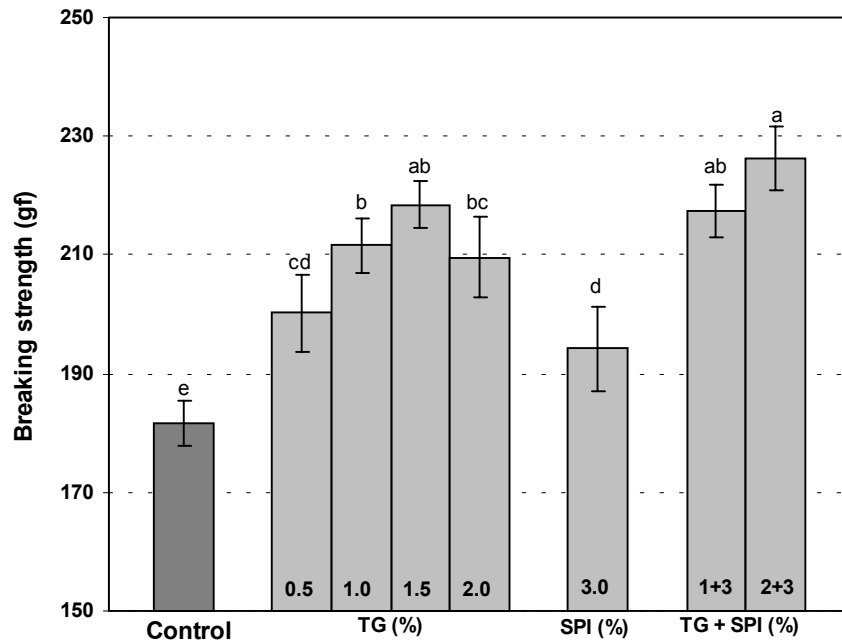
52.53. Even though 'L' value decreased in spaghetti from MACS 1967, it did not follow any particular pattern. At 2% addition of TG, 'L' value had decreased from 54.98 to 52.85. Addition of 3% SPI or TG+SPI at both the levels, also decreased the 'L' value compared to control spaghetti. The 'a' value of spaghetti samples was significantly ( $p < 0.05$ ) decreased due to TG treatment at all levels of addition. In other words, TG treatment had decreased the redness of the surface of spaghetti from both varieties. Earlier Wu and Corke (2005) also observed that brightness of dry noodles decreased due to TG treatment. Decrease in surface redness ('a' value) might be the result of a limited amount of Maillard reaction due to a decrease in the amount of available lysine because of TG reactions. Basman et al (2002a) found that the color of bread crust became lighter due to TG treatment that was attributed to the lower intensity of Maillard reaction. Furthermore, the release of ammonia during the TG-catalyzed cross-linking reaction (Motoki and Seguro, 1998) might also participate in the Maillard reaction and thus contribute slightly to the changes in color properties (Wu and Corke, 2005). On the other hand, treatment with SPI either alone or in combination with TG, significantly increased the 'a' value of spaghetti samples. However, this effect was more evident in spaghetti from PDW 274. The yellowness ('b' value) of dry spaghetti samples from both varieties decreased significantly with addition of TG. However, treatment with SPI increased the yellowness in spaghetti and compensated the decrease in yellowness due to TG treatment. In spite of this, the yellowness was still lower than that of control. Since the color characteristics reported here are the results of color reflectance measured by colorimeter, it can be contemplated that apart from the suggested chemical



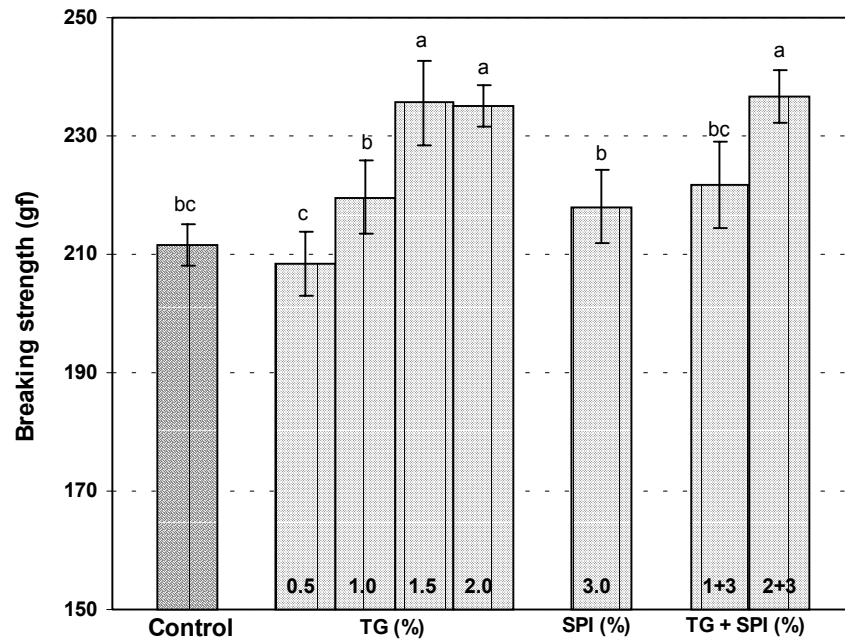
mechanisms, cross-linking by TG might also have influenced the physical structure of spaghetti thereby affecting the reflectance properties. On the whole, the negative effects of TG treatment on spaghetti color though statistically noticeable, were not visually detectable.

#### **4.4.4.4. Effects of TG, SPI and TG+SPI on the breaking strength of dry spaghetti**

Since protein is the main contributor to the strength of dry spaghetti, cross-linking of gluten proteins by TG was expected to affect this parameter. Results of the present study showed that the breaking strength of spaghetti from low protein (poor quality) variety PDW 274 significantly increased with addition of TG at all levels with maximum value observed at 1.5% level (Fig. 68). It seems that TG levels higher than 1.5% had an adverse effect on the breaking strength of spaghetti. Probably high degree of cross-linking, especially in a low protein medium, appeared to have negatively affected the integrity of gluten network and also gluten-starch interactions which are important factors contributing to strength of spaghetti. Effect of TG on the breaking strength of spaghetti from high protein (good quality) variety MACS 1967 was almost similar to that of PDW 274, except that the breaking strength increased with increasing TG level up to 1.5% (Fig. 69) and the effect of 2% TG (235.2 gf) was similar to that of 1.5% level (235.6 gf). Apparently, the high protein content of MACS 1967 had tolerated higher levels of TG compared to PDW 274 where increasing levels of TG seemed to be detrimental. Treatment with 3% SPI significantly increased the breaking strength of spaghetti from PDW 274, whereas increase in breaking strength of MACS 1967 with SPI



**Fig. 68.** Effects of TG, SPI and TG + SPI on breaking strength of dry spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of five determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.



**Fig. 69.** Effects of TG, SPI and TG + SPI on breaking strength of dry spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of five determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

addition was not statistically noticeable. Probably the soya proteins have enriched the gluten network of low protein variety PDW 274 resulting in a stronger network. Incorporation of TG+SPI combinations at both levels (1+3 and 2+3) increased the breaking strength of spaghetti samples from both the varieties. Once again, the improving effect of TG+SPI was more evident in spaghetti from poor variety PDW 274. Spaghetti from PDW 274 treated with both combinations of TG+SPI had breaking strength comparable or even higher than spaghetti from good variety MACS 1967 treated with 1+3 combination of TG+SPI. However, highest breaking strength values were recorded for spaghetti from MACS 1967 treated with 1.5 and 2% TG and also 2% + 3% TG+SPI. These studies clearly indicated that soy proteins could successfully enhance the effect of TG through cross-linking with gluten proteins resulting in an increase in the breaking strength of dry spaghetti. These findings would confirm the theory that TG reactions are able to synthesize protein conjugates by cross-linking two or more heterologous proteins (Köksel et al., 2001; Basman et al., 2002b).

#### **4.4.4.5. Effect of TG, SPI, and TG+SPI on cooking quality of spaghetti**

Effect of TG and SPI on cooking loss and cooked weight of spaghetti samples from poor variety PDW 274 and good variety MACS 1967 are shown in Table 36. Cooking loss of spaghetti from PDW 274 treated with 0.5 to 1.5% TG was lower than that of control (7.1%). Minimum cooking loss of 6.7% was seen in the sample containing 0.5% TG. On the other hand, cooking loss of spaghetti from high protein variety increased with addition of TG at all levels. Maximum cooking loss of 5.67% was seen in 1.5% TG treated sample.

**Table 36.** Cooking loss and Cooked weight of TG, SPI and TG + SPI treated spaghetti from poor variety PDW 274 and good variety MACS 1967

	PDW 274		MACS 1967	
	C. L. (%)*	C. W. (g)**	C. L. (%)*	C. W. (g)**
<b>Control</b>	7.11 ± 0.07 <sup>b</sup>	29.8 ± 0.31 <sup>a</sup>	5.35 ± 0.06 <sup>f</sup>	27.5 ± 0.27 <sup>a</sup>
<b><u>TG (%)</u></b>				
<b>0.5</b>	6.69 ± 0.10 <sup>e</sup>	29.7 ± 0.21 <sup>a</sup>	5.49 ± 0.06 <sup>ef</sup>	27.5 ± 0.35 <sup>a</sup>
<b>1.0</b>	6.91 ± 0.07 <sup>cd</sup>	28.8 ± 0.20 <sup>b</sup>	5.60 ± 0.11 <sup>de</sup>	26.4 ± 0.22 <sup>b</sup>
<b>1.5</b>	6.98 ± 0.06 <sup>bc</sup>	28.8 ± 0.28 <sup>b</sup>	5.67 ± 0.08 <sup>cd</sup>	25.9 ± 0.21 <sup>c</sup>
<b>2.0</b>	7.68 ± 0.11 <sup>a</sup>	27.8 ± 0.17 <sup>c</sup>	6.60 ± 0.05 <sup>a</sup>	25.5 ± 0.18 <sup>cd</sup>
<b><u>SPI (%)</u></b>				
<b>3.0</b>	6.81 ± 0.05 <sup>de</sup>	29.5 ± 0.31 <sup>a</sup>	5.82 ± 0.12 <sup>bc</sup>	27.5 ± 0.30 <sup>a</sup>
<b><u>TG + SPI (%)</u></b>				
<b>1 + 3</b>	6.85 ± 0.10 <sup>cde</sup>	27.2 ± 0.25 <sup>d</sup>	5.94 ± 0.07 <sup>b</sup>	25.1 ± 0.25 <sup>d</sup>
<b>2 + 3</b>	7.82 ± 0.11 <sup>a</sup>	27.1 ± 0.12 <sup>d</sup>	6.54 ± 0.10 <sup>a</sup>	24.6 ± 0.20 <sup>e</sup>

Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* C.L., Cooking loss; \*\*C. W., Cooked weight of 10g dry spaghetti.

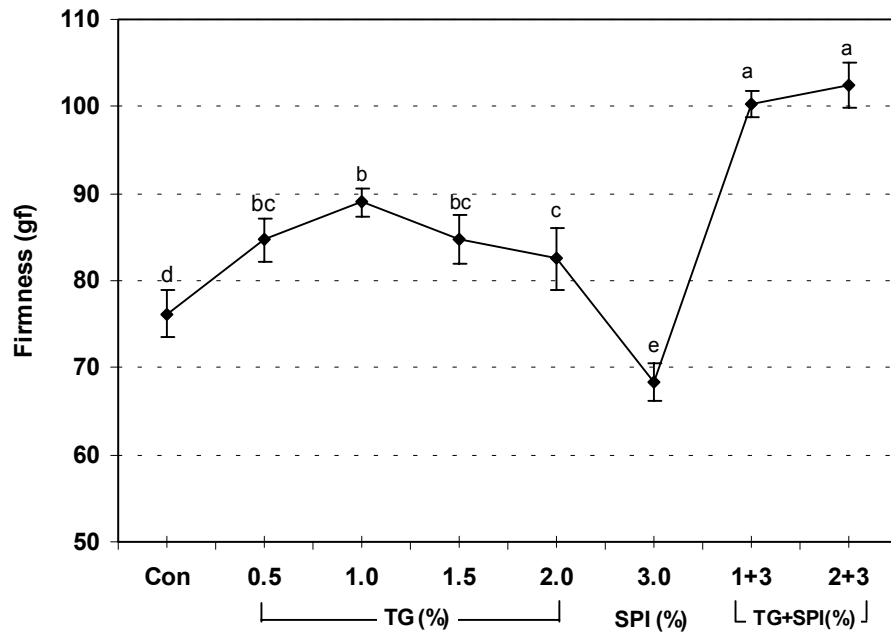
Though statistically different, 0.3% increase in cooking loss of MACS 1967 spaghetti was not noticeable. Data for cooking loss of spaghetti showed that increasing levels of TG tended to increase the cooking loss of spaghetti, irrespective of the variety. Cooking loss of both spaghetti samples with 2% TG was significantly higher than that of control. However, effect of 2% TG addition was more detrimental for spaghetti from high protein variety MACS 1967 than low protein variety PDW 274. It seems that high degree of cross-linking in gluten proteins due to addition of 2% TG would have decreased the protein-starch interactions leading to an increase in the leaching of starch components into the cooking water. Earlier Wu and Corke (2005) studying the effect of TG on white salted noodles found that cooking loss was not significantly influenced by TG treatment. However, they also reported an increasing trend in cooking loss due to increasing levels of TG from 0.5 to 2%.

Addition of 3% SPI significantly decreased the cooking loss of spaghetti from PDW 274. Probably the low protein of PDW 274 has been compensated by soya proteins resulting in a more extensive protein network capable of holding more of starch components. On the contrary, addition of 3% SPI increased the cooking loss of spaghetti from high protein variety (MACS 1967) by 0.5%. Increase in cooking loss in this spaghetti can be attributed to the leaching of some soya proteins that would have not been involved in the strong native protein network of MACS 1967 spaghetti. Haber et al (1978) reported that high water-soluble protein fraction in the soy protein may result in higher cooking loss of pasta. Combinations of TG+SPI also did not have significant positive effect on cooking loss of spaghetti except for

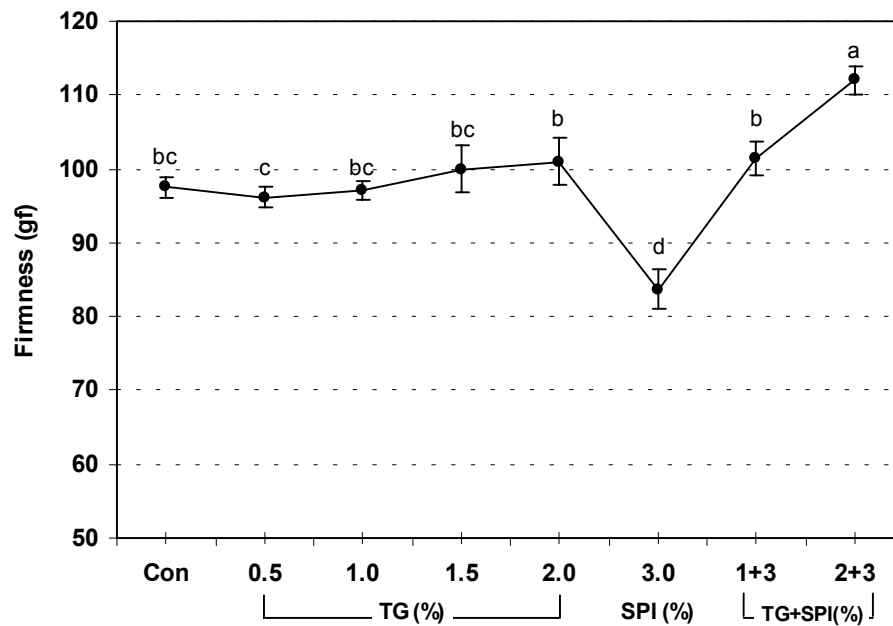
PDW 274 spaghetti treated with 1+3 combination. Though the cooking loss of spaghetti was not significantly improved by TG treatment, other cooking quality parameters should also be considered to evaluate the overall effects of TG on spaghetti cooking quality. As it was observed earlier for breaking strength, TG treatment was similarly more efficient in decreasing the cooking loss of spaghetti from low protein poor variety PDW 274.

Increasing levels of TG significantly decreased the cooked weight of spaghetti samples from both varieties. Further decrease in cooked weight was also observed in spaghetti samples treated with both combinations of TG+SPI. Addition of 3% SPI alone did not have a significant effect on cooked weight. Results of the effect of TG on cooked weight are in good agreement with the work of Wu of Corke (2005) who reported that the cooking yield (or water absorption) of white salted noodles decreased significantly with addition of TG. Since cooked weight of spaghetti is correlated with the water absorption of strands during cooking, either cross-linking of intra molecular gluten proteins or cross-linking of gluten proteins with soy proteins would perhaps create an extensive network that is able to act as a barrier against water penetration. This network therefore might prevent access of starch granules to water during cooking resulting in lesser degree of swelling.

Firmness of cooked spaghetti is another important factor in assessing cooking quality of spaghetti that is highly affected by protein quantity and quality. In the present study, it was found that firmness value increased significantly for spaghetti from PDW 274 variety with maximum value (89 gf) observed in sample containing 1% TG (Fig. 70). Further increase in levels of



**Fig. 70.** Effects of TG, SPI and TG + SPI on firmness of cooked spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of five determinations. Values with different letters are significantly different ( $p < 0.05$ ) from each other.



**Fig. 71.** Effects of TG, SPI and TG + SPI on firmness of cooked spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of five determinations. Values with different letters are significantly different ( $p < 0.05$ ) from each other.

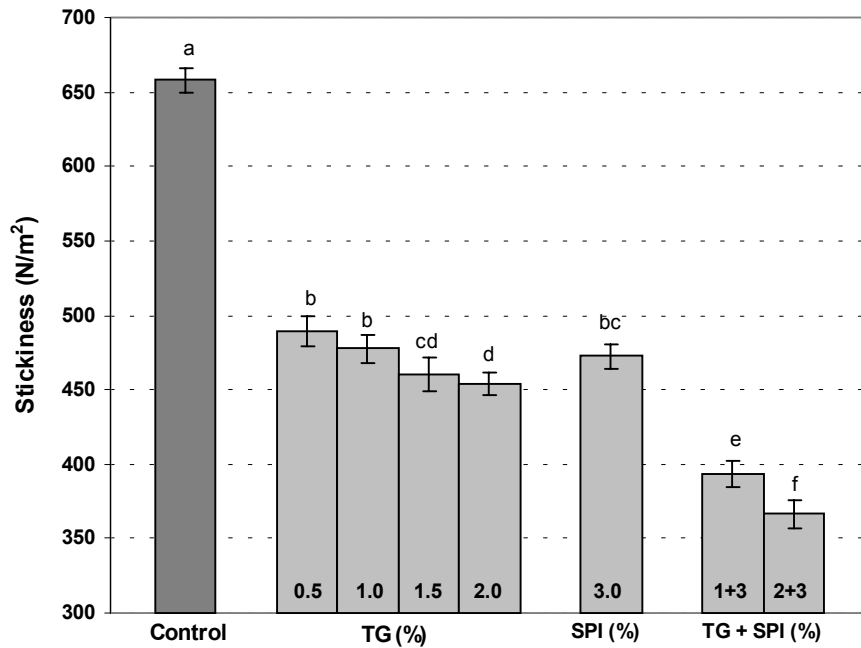
TG (1.5% and 2%) decreased the firmness. However, the values were still significantly higher than that of control. On the other hand, increasing the level of TG even though increased the firmness values of spaghetti from MACS 1967, the values were not different significantly (Fig. 71). Firmness of spaghetti from both varieties decreased significantly with addition of 3% SPI. It seems that soya proteins have acted as diluting factors preventing gluten proteins to form a uniform strong network that imparts sufficient firmness to spaghetti strands after cooking. Addition of TG along with SPI had a very significant positive effect on the firmness of spaghetti from both varieties. Results showed that while firmness of spaghetti from PDW 274 increased from 76.2 gf (control) to 102.5 gf, that of MACS 1967 increased from 97.5 gf (control) to 112.0 gf after treatment with 2 + 3% of TG + SPI, respectively. These results once again confirm possible formation of cross-links within gluten proteins and between gluten and soya proteins.

Earlier Sakamoto et al (1996) reported that the breaking strength (firmness) of boiled Chinese noodles increased correspondingly with TG concentration. However, they showed that TG concentration higher than 7.0 U/g protein decreased the breaking strength of boiled noodles. Wu and Corke (2005) also found that tensile force and hardness of cooked white salted noodles increased with increasing levels of TG. Larré et al (2000) indicated that transglutaminase cross-linked gluten appeared to be less sensitive to heat treatment than native gluten. They pointed out that the superimposition of covalent bonds to the initial gluten network would stabilize it against effects of temperature. Accordingly, Kuraishi et al (2001) reported that due to such

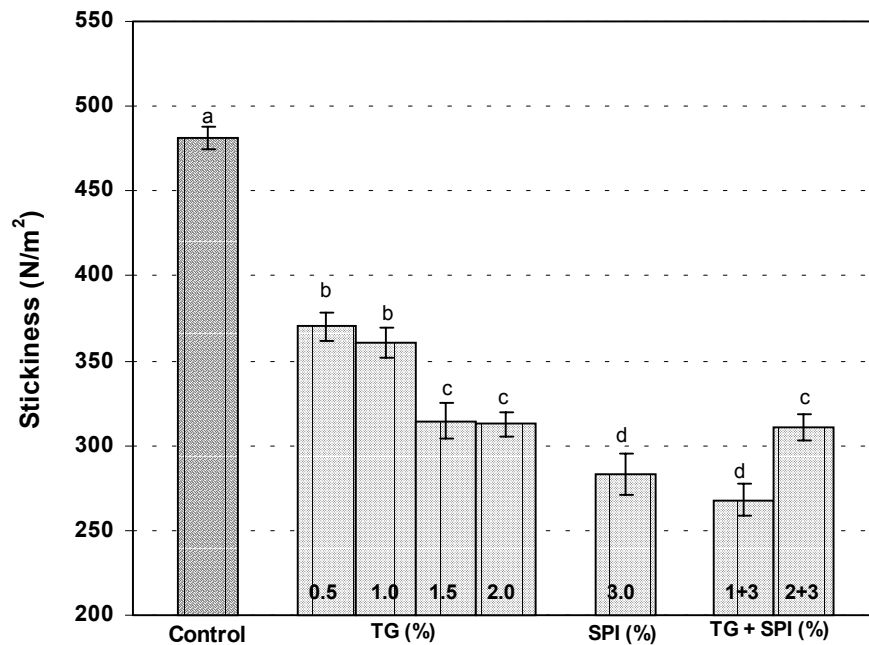


heat-stable cross-links, the firmness and elasticity of noodles are retained for longer time even after cooking. Hence, noodles treated with TG could maintain a firm texture in hot soup (Kuraishi et al., 2001).

Stickiness of treated spaghetti samples from PDW 274 and MACS 1967 are shown in Fig. 72 and 73, respectively. Stickiness of spaghetti from both varieties decreased significantly due to treatment with different TG levels, 3% SPI, and both combinations of TG+SPI. The lowest stickiness value ( $366.4 \text{ N/m}^2$ ) for spaghetti from PDW 274 was recorded for sample containing 2% + 3% combination of TG+SPI. In case of MACS 1967, the lowest stickiness value was recorded for sample containing 1% + 3% combination of TG+SPI ( $267.8 \text{ N/m}^2$ ). Decrease in surface stickiness of spaghetti samples can once again be attributed to the protein network created through cross-linking of gluten proteins or gluten and soya proteins that might be responsible in preventing leaching of the starchy material to the surface of spaghetti strands thereby decreasing the stickiness. As has already been discussed in the previous chapter, there is no relationship between cooking loss of spaghetti and surface stickiness, though starchy materials are involved in both parameters. Kuraishi et al (2001) have reported that starch granules present in dough are better held within the gluten network that is strengthened by the addition of transglutaminase and therefore would be responsible for the surface of noodles becoming less sticky and with a reduction in bulkiness of the cooked noodles.



**Fig. 72.** Effects of of TG, SPI and TG + SPI on stickiness of cooked spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of three determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

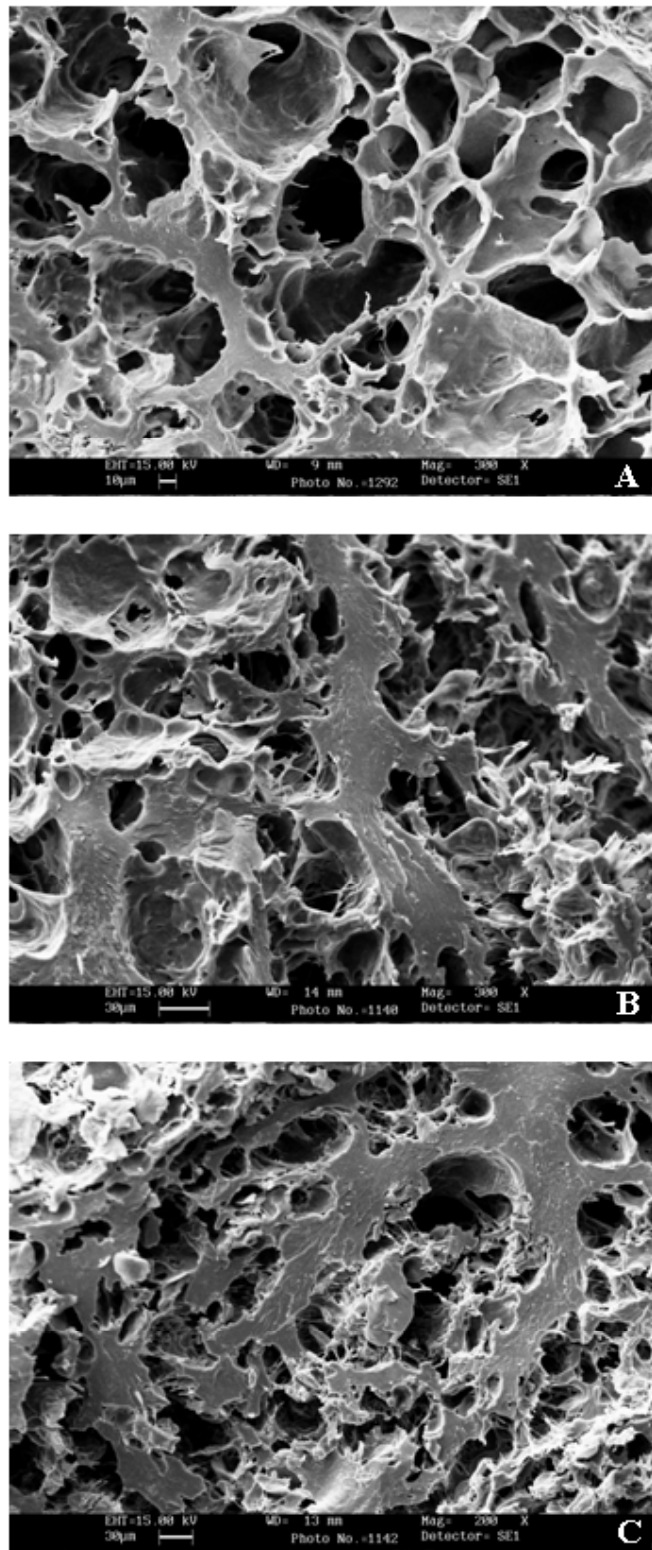


**Fig. 73.** Effects of TG, SPI and TG + SPI on stickiness of cooked spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of three determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other.

The results of spaghetti cooking quality clearly showed that TG is able to catalyze the formation of cross-links between gluten proteins and soya proteins to improve the overall quality of the product. Köksel et al (2001) in their study on rheological properties of wheat flour found that SPI did not significantly influence the action of TG. However, they pointed out that their study was mainly based on oscillatory measurement and therefore, studies to cover other rheological tests and actual baking test (bread) are warranted. Basman et al (2002b) studying the SDS-PAGE patterns of TG treated wheat, soy and wheat soy blends, showed the possibility for cross-linking between wheat and soy proteins by TG enzyme. On the other hand, Bauer et al (2003b) reported that the effect of TG depends strongly on the processing conditions. This has been supported by work of Lauber et al (2001, 2003) who found that whey proteins are cross-linked to a high extent under high hydrostatic pressure, whereas under atmospheric pressure almost no cross-linking was obtained.

#### **4.4.5. Effect of TG on microstructure of spaghetti**

Scanning electron microscopy (SEM) of cross-section of cooked and freeze-dried spaghetti samples treated with different levels of TG clearly showed that protein network had become much tighter compared to control samples. Representative micrographs in Fig. 74 show that with increasing levels of TG, the protein network appears to be thicker and stronger. However, the strengthened protein network was not uniformly distributed in whole area of cross-section, particularly at higher level (2%) of TG addition and this might be due to the high degree of cross-linking and compactness of



**Fig. 74.** Scanning electron micrographs of cross section (below surface) of cooked spaghetti from PDW 274. (A) Control; (B) Treated with 1% TG; (C) Treated with 2% TG.

the gluten proteins. This can confirm as to why 2% TG addition did not show further improvement in some of the spaghetti properties such as breaking strength, cooking loss, and firmness. Bauer et al (2003b) reported the formation of  $\epsilon$ -( $\gamma$ -Gln)-Lys cross-links with the addition of TG that affected the structure and therefore the viscoelastic properties of the gluten network. This was reflected in a decrease in the extensibility and an increase in the resistance to extensibility when TG was added to flour. Sakamoto et al (1996) also reported a stronger protein network for uncooked Chinese noodles after treatment with TG when viewed under SEM. Similarly, Wu and Corke (2005) using SEM data, observed some improvements in protein network of white dry salted noodles due to TG treatment. They also reported that the obvious cracks in cooked noodles were less apparent in TG treated noodles.

#### **4.4.6. Effect of Lipase (Li), Distilled Glycerol Monostearate (DGMS), and Li + DGMS on pasting properties of semolina**

Pasting properties of powdered semolina samples from durum varieties MACS 1967 and PDW 274 mixed with different levels of microbial lipase (25, 50, 100, 150, 200 ppm), 0.5% (w/w) DGMS, and a combination of lipase + DGMS (50 ppm + 0.5%) were determined using a micro visco-amylograph. Different levels of lipase did not show any marked effect on the pasting properties of semolina from both the varieties. However, the effects of DGMS and Li+DGMS on semolina pasting properties were significant. Onset gelatinization temperature of semolina from PDW 274 (64.1 °C) increased to 64.9 and 65.1 °C, and that of MACS 1967 (66.3 °C) increased to 67.0 and 67.5 °C due to addition of DGMS and Li+DGMS, respectively. While peak

viscosity decreased, breakdown and setback viscosities increased due to treatment with DGMS and Li+DGMS. Peak viscosity of PDW 274 (748 B.U.) decreased to 710 and 715 B.U. and that of MACS 1967 (830 B.U.) decreased to 785 and 792 B.U., after treatment with 0.5% DGMS and combination of Li+DGMS, respectively. DGMS and Li+DGMS treatments significantly increased the breakdown viscosity of PDW 274 (90 B.U.) to 104 and 125 B.U., and that of MACS 1967 (115 B.U.) to 138 and 159 B.U., respectively. On the other hand, setback viscosity of PDW 274 (530 B.U.) increased to 632 and 668 B.U., respectively. Matsuo et al (1986) also found that monoglycerides increased setback viscosity significantly. Medcalf et al (1968) reported that the polar lipids–starch complex would tend to reduce or retard the rate of hydration of the starch molecules in amorphous regions of the granules resulting in a lower maximum peak height. Krog (1973) also reported a significant increase in the pasting temperature of wheat starch with addition of 0.5% monoglycerides. Negligible effects of lipase on semolina pasting properties might be due to the highly diluted system used in the study of pasting properties that might have affected the enzyme activity. Therefore, semolina pasting properties under the influence of lipase treatment might not be able to predict the behavior of this enzyme during spaghetti processing. Moreover, it has been found that lipids in pasta are less extractable than in semolina suggesting that under the mechanical action of the extrusion screw, lipids undergo chemical changes or are complexed or both (Fabriani et al., 1968).

#### **4.4.7. Effect of lipase, DGMS and Li+DGMS on color characteristics of dry spaghetti**

To study the effect of microbial lipase on spaghetti quality and cooking quality, three levels of enzymes, namely, 50, 100, and 150 ppm (equal to 15, 30, and 45 KLU/kg semolina) were selected for spaghetti production. These levels were in the range reported in the literature for noodles and bread wheat pasta (Si and Lustenberger, 2002) and also higher than that proposed (5-30 KLU/kg flour) by the manufacturers (Noopazyme). DGMS at 0.5% (w/w) level was used alone and in combination with lipase (50 ppm) to study their effect on spaghetti quality. Results of the above treatments on color characteristics of dry spaghetti from PDW 274 and MACS 1967 are shown in Tables 37 and 38, respectively. Effect of lipase on the color characteristics of spaghetti from both varieties was almost similar. Brightness ('L' value) and yellowness ('b' value) decreased, whereas 'a' (redness-greenness) value increased due to lipase treatment. It has been shown that lipase increases the brightness of noodles and also reduces the darkening rate during storage of raw noodles (Si and Lustenberger, 2002; Forman and Lustenberger, 2001). Same researchers also found that the surface of the lipase-treated noodles was much smoother compared to the surface of the control noodles, which would have been the reason for increase in surface brightness of noodles. However, in the present context, decrease in 'yellowness' of spaghetti can perhaps be attributed to the bleaching potential of lipase (Mercier and Gélinas, 2001). Subjective and objective observations showed that the addition of 0.5% DGMS did not have a substantial effect on the color characteristics of spaghetti from both durum varieties.

**Table 37.** Effects of lipase, DGMS and Li + DGMS on color characteristics\* of dry spaghetti from poor variety PDW 274.

	<b>L</b>	<b>a</b>	<b>b</b>
<b>Control</b>	55.58 ± 0.18 <sup>a</sup>	-0.59 ± 0.08 <sup>b</sup>	22.64 ± 0.19 <sup>a</sup>
<b><u>Lipase (ppm)</u></b>			
<b>50</b>	50.40 ± 0.74 <sup>c</sup>	-0.08 ± 0.03 <sup>c</sup>	19.28 ± 0.35 <sup>d</sup>
<b>100</b>	50.74 ± 0.30 <sup>c</sup>	-0.10 ± 0.05 <sup>c</sup>	19.62 ± 0.18 <sup>d</sup>
<b>150</b>	50.80 ± 0.20 <sup>c</sup>	-0.08 ± 0.04 <sup>c</sup>	19.20 ± 0.22 <sup>d</sup>
<b><u>DGMS (%)</u></b>			
<b>0.5</b>	53.17 ± 0.26 <sup>b</sup>	-0.85 ± 0.18 <sup>a</sup>	22.11 ± 0.05 <sup>b</sup>
<b><u>Li + DGMS**</u></b>			
<b>50 + 0.5</b>	51.11 ± 0.29 <sup>c</sup>	-0.2 ± 0.03 <sup>c</sup>	20.20 ± 0.12 <sup>c</sup>

Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* L: brightness; a: redness-greenness; b: yellowness.

\*\* Lipase (50 ppm) + DGMS (0.5%)



**Table 38.** Effects of lipase, DGMS and Li + DGMS on color characteristics\* of dry spaghetti from good variety MACS 1967.

	<b>L</b>	<b>a</b>	<b>b</b>
<b>Control</b>	54.98 ± 0.34 <sup>b</sup>	-0.53 ± 0.07 <sup>c</sup>	21.81 ± 0.25 <sup>a</sup>
<b><u>Lipase (ppm)</u></b>			
<b>50</b>	52.76 ± 0.20 <sup>cd</sup>	-0.21 ± 0.10 <sup>d</sup>	17.90 ± 0.32 <sup>d</sup>
<b>100</b>	52.34 ± 0.37 <sup>d</sup>	-0.17 ± 0.05 <sup>d</sup>	18.40 ± 0.21 <sup>c</sup>
<b>150</b>	52.66 ± 0.11 <sup>cd</sup>	-0.20 ± 0.08 <sup>d</sup>	18.33 ± 0.17 <sup>cd</sup>
<b><u>DGMS (%)</u></b>			
<b>0.5</b>	55.96 ± 0.42 <sup>a</sup>	-1.41 ± 0.12 <sup>a</sup>	21.36 ± 0.24 <sup>a</sup>
<b><u>Li + DGMS**</u></b>			
<b>50 + 0.5</b>	53.20 ± 0.30 <sup>c</sup>	-1.08 ± 0.09 <sup>b</sup>	20.23 ± 0.19 <sup>b</sup>

Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* L: brightness; a: redness-greenness; b: yellowness.

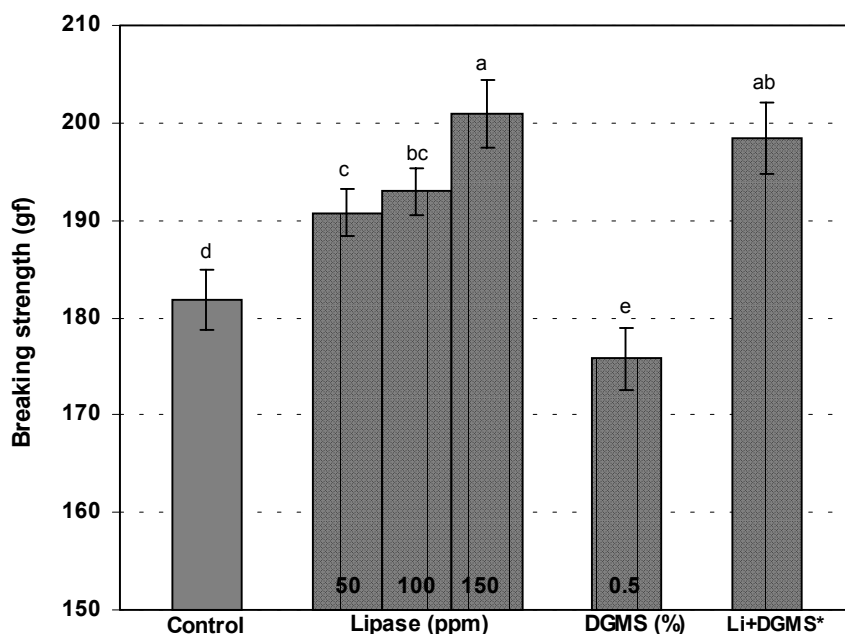
\*\* Lipase (50 ppm) + DGMS (0.5%)

#### **4.4.8. Effect of lipase, DGMS, and Li+DGMS on breaking strength of dry spaghetti**

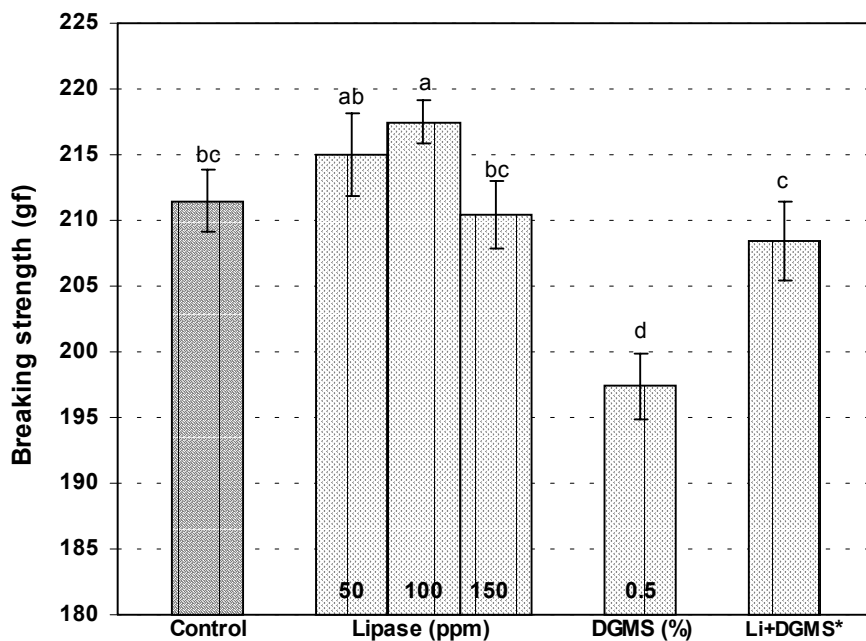
Breaking strength of dry spaghetti samples increased with addition of lipase and continued to increase with each increase in the level added (Figures 75 and 76). These results were more evident in spaghetti from low protein variety PDW 274. Treatment with 0.5% DGMS lowered the breaking strength of spaghetti significantly. Addition of Li+DGMS combination increased the breaking strength of spaghetti from PDW 274, while it had no effect on spaghetti from MACS 1967. Earlier studies on the dynamic rheological properties have shown an increase in gluten strength due to treatment with lipase, which can be useful in bread making (Jakobsen and Si, 1995). However, the nature of cross-links in the gluten network is yet to be elucidated (Si and Lustenberger, 2002). Therefore, the increase in spaghetti breaking strength might be attributed to strengthening of gluten proteins due to lipase treatment.

#### **4.4.9. Effect of lipase, DGMS, and Li+DGMS on cooking quality characteristics of spaghetti**

Cooking loss of spaghetti from PDW 274 decreased significantly after treatment with different levels of lipase, whereas lipase did not substantially affect the cooking loss of spaghetti from MACS 1967 (Table 39). The effect of 0.5% DGMS and Li+DGMS was similar to that of lipase for spaghetti from PDW 274 in that there was significant decrease in the cooking loss. On the other hand, there was a slight increase in the cooking loss of spaghetti from MACS 1967. All the above treatments decreased the cooked weight of spaghetti from both durum varieties. Though the mechanism of the beneficial



**Fig. 75.** Effects of lipase, DGMS, and Li + DGMS on breaking strength of dry spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of five determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).



**Fig. 76.** Effects of lipase, DGMS, and Li + DGMS on breaking strength of dry spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of five determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).

effect of lipase in pasta or noodles is not yet fully elucidated, it may be due to modification of both the protein and the starch fractions (Si and Lustenberger, 2002). On the other hand, results from differential scanning calorimeter (DSC) and light microscopy studies have confirmed an increased formation of amylose–lipid complexes as a result of the lipase treatment (Forman and Lustenberger, 2001). These water insoluble complexes which are reported to be formed by monoglycerides (such as DGMS) too (Larson, 1982; Eliason and Krog, 1985), may retard the water uptake by starch granules resulting in less swollen granules and may also reduce the leaching of amylose during cooking resulting in lowered cooked weight and cooking loss, respectively. However, the above results have been partly confirmed in bread and noodle making which are made from aestivum wheat flour with starch and protein properties different from those of durum wheat semolina, which is the raw material for pasta preparation. Pasta dough is not a fully developed dough which is made in a short period of 10-15 min, whereas in the case of bread, a completely developed dough is formed, and in the case of noodle the sheeting and kneading processes can affect more the reactivity of components. Earlier, Grant et al (1993) found that the addition of monoglyceride decreased the cooked weight of spaghetti, whereas cooking loss remained unchanged. Matsuo et al (1986) reported a lower spaghetti cooking loss after treatment with 0.5% monoglyceride; however, the decrease in cooking loss was not significant.

**Table 39.** Cooking loss and Cooked weight of lipase, DGMS and Li + DGMS treated spaghetti from poor variety PDW 274 and good variety MACS 1967

	PDW 274		MACS 1967	
	C. L. (%)*	C. W. (g)*	C. L. (%)*	C. W. (g)*
<b>Control</b>	7.11 ± 0.07 <sup>a</sup>	29.8 ± 0.31 <sup>a</sup>	5.35 ± 0.06 <sup>d</sup>	27.5 ± 0.27 <sup>a</sup>
<b><u>Lipase (ppm)</u></b>				
<b>50</b>	6.56 ± 0.11 <sup>c</sup>	29.1 ± 0.15 <sup>b</sup>	5.40 ± 0.09 <sup>cd</sup>	25.6 ± 0.21 <sup>d</sup>
<b>100</b>	6.54 ± 0.05 <sup>c</sup>	29.0 ± 0.30 <sup>b</sup>	5.49 ± 0.06 <sup>cd</sup>	26.4 ± 0.17 <sup>c</sup>
<b>150</b>	6.49 ± 0.08 <sup>c</sup>	28.8 ± 0.31 <sup>bc</sup>	5.58 ± 0.15 <sup>bc</sup>	26.7 ± 0.34 <sup>bc</sup>
<b><u>DGMS (%)</u></b>				
<b>0.5</b>	6.41 ± 0.12 <sup>c</sup>	29.0 ± 0.18 <sup>b</sup>	5.72 ± 0.10 <sup>ab</sup>	27.0 ± 0.15 <sup>b</sup>
<b><u>Li + DGMS**</u></b>				
<b>50 + 0.5</b>	6.79 ± 0.10 <sup>b</sup>	28.5 ± 0.25 <sup>c</sup>	5.81 ± 0.11 <sup>a</sup>	26.8 ± 0.18 <sup>bc</sup>

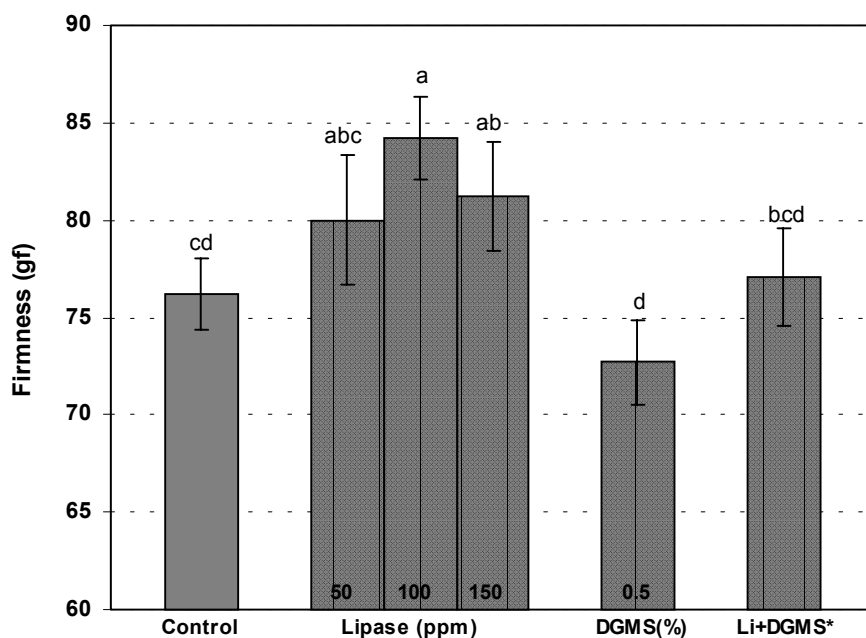
Data are expressed as mean ±SD of three determinations. Means in the same column followed by different letters are significantly different (p<0.05).

\* C.L., Cooking loss; C. W., Cooked weight of 10g dry spaghetti.

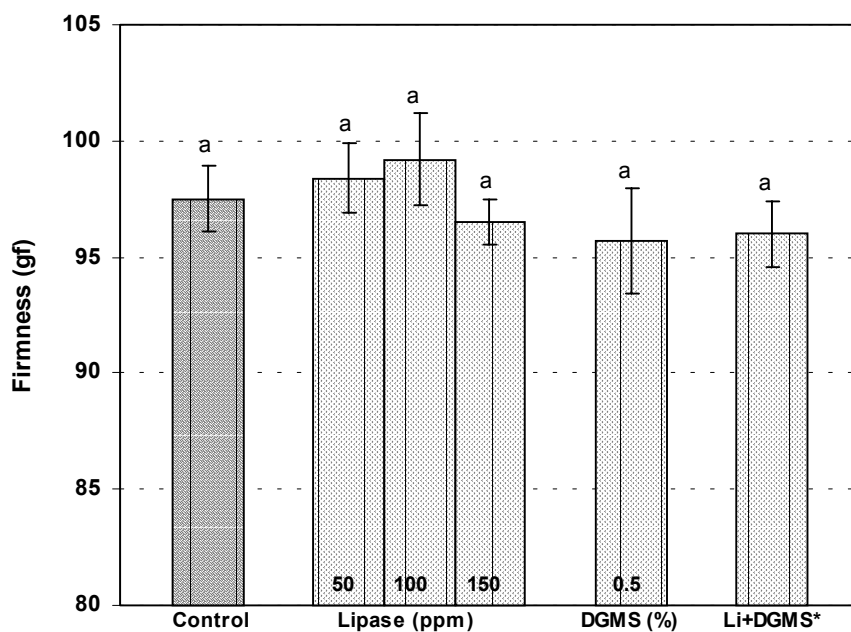
\*\* Lipase (50 ppm) + DGMS (0.5%)

Firmness of cooked spaghetti from both durum varieties increased significantly with addition of lipase at all three levels (Figures 77 and 78). However, increase in firmness was less significant in spaghetti from MACS 1967 but more evident in PDW 274 spaghetti. Neither DGMS nor Li+DGMS were effective in increasing the spaghetti firmness. Si and Lustenberger (2002) reported that lipase increased the firmness of aestivum-wheat pasta and the pasta sample treated with 200 ppm of lipase had a firmness value close to that of durum wheat pasta. Three alternative explanations for increasing spaghetti firmness due to lipase addition might be: (i) Increased formation of amylose-lipid complexes, (ii) less swelling of starch granules, particularly in the center of cooked strands, and (iii) strengthening of the gluten network.

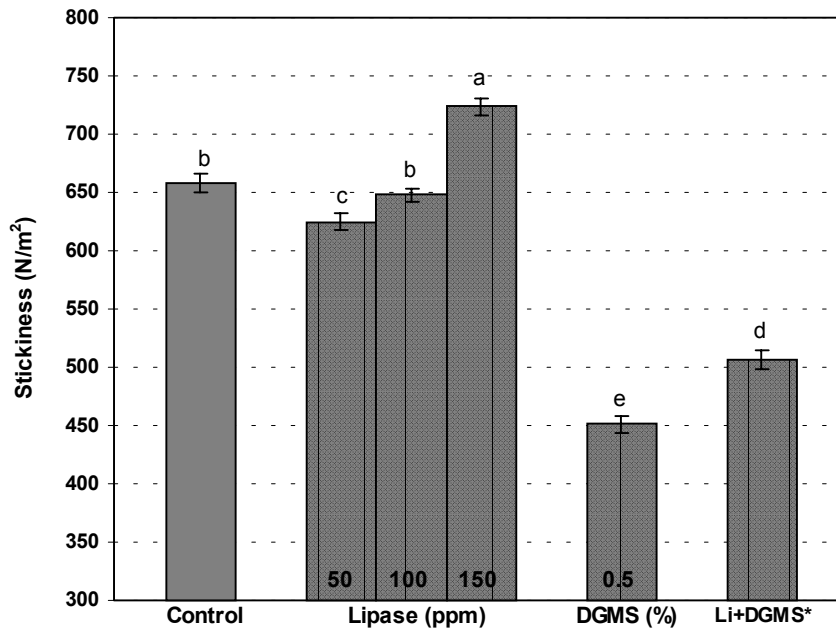
Surface stickiness values of treated spaghetti samples from PDW 274 and MACS 1967 are shown in Figures 79 and 80, respectively. Treatment with 50 and 100 ppm of lipase decreased the stickiness of cooked spaghetti samples from both durum varieties. However, the decrease in stickiness was more evident in spaghetti from MACS 1967. Addition of higher level of lipase (150 ppm) increased the stickiness of PDW 274 spaghetti more than that of control, while it had no effect on stickiness of spaghetti from MACS 1967. DGMS and Li+DGMS were also effective in decreasing the stickiness, and their effect was more evident in spaghetti from PDW 274. Si and Lustenberger (2002) reported that when lipase was used at lower level (~30ppm) the surface stickiness of pasta made from aestivum wheat decreased. But when the lipase concentration was increased to a higher level (~200 ppm), there



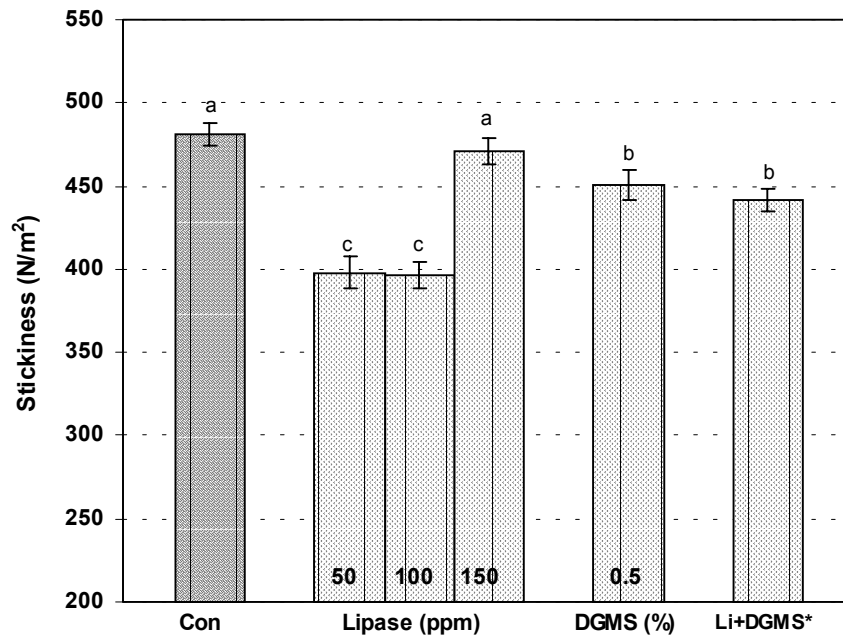
**Fig. 77.** Effects of lipase, DGMS and Li + DGMS on firmness of cooked spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of five determinations. Values with different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).



**Fig. 78.** Effects of lipase, DGMS and Li + DGMS on firmness of cooked spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of five determinations. Values with different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).



**Fig. 79.** Effects of lipase, DGMS, and Li + DGMS on stickiness of cooked spaghetti from poor variety PDW 274. Data are expressed as mean  $\pm$ SD of three determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).



**Fig. 80.** Effects of lipase, DGMS, and Li + DGMS on stickiness of cooked spaghetti from good variety MACS 1967. Data are expressed as mean  $\pm$ SD of three determinations. Bars carrying different letters are significantly different ( $p < 0.05$ ) from each other. \*Li + DGMS = lipase (50 ppm) + DGMS (0.5%).



was an increase in the surface stickiness. However, this value was still lower than that obtained for the control sample. On the other hand, the lowest value for stickiness in these experiments was still four-fold higher than that recorded for control pasta prepared from durum wheat semolina. Similarly, Matsuo et al (1986) and Grant et al (1993) had found a significant decrease in stickiness of pasta treated with monoglycerides. Amylose on the surface of cooked spaghetti is considered as a contributing factor to surface stickiness (Dexter et al., 1985). A decrease in the surface stickiness of pasta can be attributed to the formation of an amylose-lipid complex or an amylose-monoglyceride complex formed as a result of treatment with lipase or DGMS, respectively, and this would have restricted the leaching of amylose molecules to the surface of spaghetti.

#### **4.4.10. Conclusions**

The results presented in this study clearly demonstrated the effects of microbial TG on the solubility and also on the SDS-PAGE patterns of durum wheat proteins. Protein cross-linking reaction catalyzed by TG resulted in changes in dough properties, dry spaghetti quality, cooking quality characteristics and microstructure of cooked spaghetti. However, the quality improvements were more evident in spaghetti from low protein (poor quality) variety PDW 274 than in high protein (good quality) variety MACS 1967. The results also showed the ability of TG in the formation of heterologous polymers between soya proteins (rich in lysine) and durum wheat proteins (rich in glutamine) to improve the quality of spaghetti samples. It is worthwhile to mention here that SPI which contains high concentration of good quality

proteins, in addition to providing sites for TG activity, can also improve the nutritional quality of spaghetti, especially enriching it with essential amino acid lysine which is limiting in wheat proteins. Furthermore, protein cross-linking by TG was very effective in the presence of relatively very low water content in spaghetti dough and also a very short reaction time (15-20 min). Therefore, it is possible to utilize TG in pasta production to improve its overall quality, without any changes in the usual manufacturing processes followed and the equipments used.

Studies on the effect of fungal lipase on semolina properties and spaghetti quality showed the ability of this enzyme in improving the breaking strength of dry spaghetti and also in increasing the firmness and reducing the stickiness of cooked spaghetti. Lipase treatment also significantly decreased the cooked weight of spaghetti. Lipase also decreased the cooking loss of spaghetti from low protein variety PDW 274. On the other hand, lipase essentially had no effect on the cooking loss of spaghetti from the high protein variety MACS 1967. Lipase treatment also did not show any improvement in spaghetti color characteristics. Based on these results, it can be concluded that the beneficial effects of lipase on pasta might not only be due to the formation of amylose–lipid complexes but also due to its strengthening effect on gluten proteins.

Durum wheat is the raw material of choice for the production of pasta products, mainly due to its higher protein content, higher levels of carotenoid pigments, harder and more vitreous kernels, compared to those of aestivum wheat. Hard, tough, and horny endosperm of durum wheat facilitates a yield of good quality semolina and less amount of flour during milling process. Semolina is the granular product of durum wheat milling, which is used for the production of spaghetti and other pasta products. Physical characteristics of durum wheat kernel such as test weight, 1000-kernel weight, kernel size, and the degree of kernel vitreousness, are known to be important in milling process for production of semolina with desired properties suitable for pasta production. On the other hand, semolina quality is judged by several common factors such as moisture content, granulation, color characteristics, yellow pigment, ash, and protein content.

More than 50 percent of world durum wheat production is converted into different pasta products such as spaghetti, macaroni, vermicelli etc. These products are nutritional and healthy and can be prepared in variety of ways. They are favored by consumers for their ease of transportation and preparation, palatability, and long shelf life. Therefore, pasta products are becoming increasingly popular not only worldwide but also with the Indian consumers. Spaghetti that is the solid cylindrical form of pasta, is the most popular and common variety among the hundreds of shapes of pasta products and is the preferred pasta shape for laboratory evaluation of pasta.

Spaghetti made from durum wheat varieties of superior quality results in a bright yellow color, which is retained after cooking, firm texture and its resistant to surface disintegration and stickiness. However, not all durum

wheat semolina produces spaghetti of good cooking quality; many variables are involved in spaghetti manufacturing and their role is not completely understood. Quantity and quality of protein in durum wheat and semolina are known to be important factors for the cooking quality of spaghetti. Apart from proteins, role of starch in pasta cooking quality has been better understood in recent years. Besides wheat components, the physical characteristics of durum wheat and semolina might influence the pasta quality directly or indirectly. The bright yellow color of pasta products, rather than cooking behavior and taste, is reported to be one of the most important considerations in assessing durum wheat quality. However, a high level of yellow pigments in durum wheat and semolina does not guarantee a high yellow color in pasta, because pasta yellowness and pigment loss during processing are mainly affected by enzymes such as lipoxygenase (LOX), peroxidase (POD), and polyphenol oxidase (PPO) activities.

Wheat production in India including the durum wheat has increased significantly during the past several decades as a result of the green revolution. Recently, several new durum varieties have been developed by different Indian Agricultural Research Institutions and Universities. However, there is little in-depth scientific research work available regarding the physico-chemical characteristics of Indian durum wheats and their relation to semolina milling and spaghetti-making quality. This research work was carried out to study various properties of selected Indian durum varieties, their semolina milling potential, and their suitability for pasta production. Latter part of the work focused on quality improvement of spaghetti using high temperature drying, and additives such as microbial transglutaminase, soya protein isolate,

microbial lipase, and surfactant (DGMS). This investigation was planned to cover the following three main objectives:

1. To study the physico-chemical properties of Indian durum wheat
2. To study the semolina milling quality of Indian durum wheat
3. To study the spaghetti-making quality of Indian durum wheat

**The salient findings of this investigation were:**

- \* Protein content of 14 Indian durum wheat ranged from 10.7% to 15.9% with an average of 13.6%. Their ash content was between 1.38-2.14% with an average of 1.95%, and their yellow pigment content varied from 3.8 ppm to 7.2 ppm with average of 5.2 ppm.
- \* LOX activity in 14 Indian durum varieties showed lot of variations among the varieties and ranged from 1.4 to 6.9 U/g.
- \* POD activity showed lot of variations among wheat varieties and varied from 269 to 1010 U/g.
- \* PPO activity of Indian durum varieties was between 53.8 and 78.3 U/g with a quite less variation compared to LOX and POD.
- \* Protease activity of Indian durum varieties was in low range of 1.1 – 2.8 U/g except for variety MACS 1967, which had an activity of 5.1 U/g.
- \* Four HMW-GS compositions of 6+8, 7+8, 13+16, and 20 were identified on SDS-PAGE pattern of 14 Indian durum varieties.
- \* HMW-GS 7+8 had the highest frequency among the varieties studied.
- The optimum conditions for semolina milling were found to be:

Gap spaces of 0.4 and 0.2 mm for break rolls B1 and B3, respectively, and tempering moisture of 17% for 18 h.

- ✦ Six selected Indian durum wheat varieties studied had test weight of 79.75 to 84.0 kg/hl, and 1000-kernel weight of 40.3 to 48.4 g.
- ✦ Varieties DWR 2006 and MACS 1967 exhibited the largest kernels among varieties studied.
- ✦ Six Indian durum varieties had vitreousness of 86.6 to 100% with average of 93.5%.
- ✦ Variety MACS 1967 exhibited the hardest kernels among the varieties studied.
- ✦ Semolina milling yield of six durum varieties ranged from 57.3 to 63.7% with an average of around 61%.
- ✦ Variety WH 896 with the lowest 1000-kernel weight, vitreousness, and hardness, and with the smallest kernels showed the lowest semolina milling yield.
- ✦ Correlation coefficient of 0.81\*\* and 0.61\*\* were found between 1000-kernel weight and vitreousness, respectively, with semolina milling yield.
- ✦ Six semolina samples had ash content between 0.79 to 0.86%.
- ✦ Semolina from variety MACS 1967 had the highest amount of wet gluten (34.4%), while semolina from PDW 274 had the lowest amount (25.2%).
- ✦ Variety PDW 274 showed the lowest content of acetic acid insoluble proteins (4.3%).

- ✦ Scanning electron micrographs of semolina from WH 896 showed a less tight and compact structure compared to other varieties.
- ✦ Scanning electron micrographs of semolina from PDW 274 showed larger starch granules, in which majority of them were not covered by protein matrix.
- ✦ There was a significant negative correlation ( $r = -0.68^{**}$ ) between semolina wet gluten and dough development time of farinograph.
- ✦ A positive correlation ( $r = 0.69^{**}$ ) was found between semolina wet gluten and maximum consistency of farinograph.
- ✦ Total protein of semolina was highly negatively correlated ( $r = -0.81^{**}$ ) with dough development time, and highly positively correlated ( $r = 0.88^{**}$ ) with maximum consistency.
- ✦ Results of subjective and objective evaluations showed that variety MACS 1967 was excellent and PDW 274 was poor for spaghetti production.
- ✦ A significant negative correlation ( $r = -0.79^{**}$ ) was found between semolina wet gluten and cooking loss of spaghetti.
- ✦ Wet gluten of semolina and firmness of spaghetti samples were highly correlated ( $r = 0.79^{**}$ ).
- ✦ Firmness of cooked spaghetti was negatively correlated to cooking loss ( $r = -0.91^{**}$ ) and stickiness ( $r = -0.64^{**}$ ).
- ✦ Results of micro visco-amylograph showed a high reverse correlation ( $r = -0.84^{**}$ ) between semolina peak viscosity and stickiness of spaghetti.
- ✦ Results of SDS-PAGE analysis showed that a protein with molecular weight around 45 kDa was absent in poor durum variety PDW 274.

- ✦ RP-HPLC revealed absence of a peak designated as GliPK 36-37 in poor variety PDW 274.
- ✦ SDS-PAGE analysis of albumins, globulins, gliadins, and glutenins of good variety MACS 1967 and poor variety PDW 274 showed that there were no significant differences between albumins and globulins fractions, while their gliadins and glutenins fractions were different significantly.
  
- High temperature (HT) drying significantly improved retention of yellow pigment and also the color characteristics of spaghetti.
- Scanning electron microscopy of HT-dried spaghetti showed a smoother surface with a more continuous protein matrix compared to that of LT-dried spaghetti, indicative of significant improvement in microstructure, especially in spaghetti from poor variety PDW 274.
- HT drying process significantly affected the starch pasting properties of spaghetti compared to that of LT-dried spaghetti.
- HT drying process substantially affected the distribution and solubility of albumins, globulins, gliadins, and glutenins fractions. However, the gliadin fraction was less affected.
- Cooking loss, firmness, and stickiness of HT-dried spaghetti samples, especially spaghetti from poor variety PDW 274, were significantly improved compared to LT-dried spaghetti.
- Effect of different cooking times (4-20 min) on cooking quality parameters, microstructure, and pasting properties indicated clear differences between spaghetti from good and poor durum varieties and also HT- and LT-dried spaghetti samples.



- ⊕ Microbial transglutaminase (TG) significantly decreased the solubility of gluten proteins probably due to formation of high molecular weight cross-linked proteins.
- ⊕ SDS-PAGE analysis showed that increasing levels of TG progressively decreased the intensity of protein band with MW around 66 kDa, and concomitantly increased the degree of streaking due to formation of high molecular weight polymers.
- ⊕ Results of farinograph studies showed that increasing levels of TG increased the water absorption of semolina dough.
- ⊕ Micro visco-amylgraph studies showed that TG significantly increased the peak viscosity of semolina samples.
- ⊕ TG significantly increased the breaking strength of dry spaghetti, and firmness of cooked spaghetti samples.
- ⊕ Cooked weight and stickiness of cooked spaghetti samples significantly decreased due to treatment with TG.
- ⊕ Combination of TG+SPI (soy protein isolate) significantly increased dry spaghetti strength and cooked spaghetti firmness, and decreased stickiness of cooked spaghetti.
- ⊕ Quality improvement of spaghetti due to TG or TG+SPI treatment was more evident for spaghetti from low protein-poor variety PDW 274.
- ⊕ Microbial lipase increased the breaking strength of dry spaghetti and firmness of cooked spaghetti.

**From these studies, it can be concluded that:**

- ☑ Indian durum varieties compared well, in one property or the other, in their physicochemical, biochemical, semolina milling, and spaghetti-making properties, with some of the well-known Canadian and Italian durum varieties reported in literature.
- ☑ Quality improvement of spaghetti from high temperature drying process is indicative of the effect of not only protein but also starch and starch-protein interactions.
- ☑ Protein cross-linking by TG was very effective in the presence of relatively very low water content in spaghetti dough and also a very short reaction time (15-20 min). Therefore, it is possible to utilize TG in pasta production to improve its overall quality without any changes in the usual manufacturing processes followed and the equipments used.
- ☑ Addition of SPI along with TG not only enhances the effect of TG through formation of heterologous polymers between wheat proteins and soya proteins, it can also improve the nutritional quality of spaghetti, especially enriching it with essential amino acid lysine.
- ☑ The beneficial effects of lipase on pasta might not only be due to the formation of amylose-lipid complexes but also due to its strengthening effect on gluten proteins.

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## **Publications**

1. **Aalami, M.**, Leelavathi, K., & Prasada Rao, U.J.S. (2007). Spaghetti making potential of Indian durum wheat varieties in relation to their protein, yellow pigment and enzyme contents. *Food Chemistry*, 100(3), 1243-1248.
2. **Aalami, M.**, Prasada Rao, U.J.S., & Leelavathi, K. (2006). Physicochemical and biochemical characteristics of Indian durum wheat varieties: Relationship to semolina milling and spaghetti making quality. *Food Chemistry*, (doi: 10.1016/j.foodchem.2006.06.052).
3. **Aalami, M.**, Leelavathi, K. Effects of microbial transglutaminase on semolina dough properties and spaghetti quality. (To be communicated).

## **Presentations**

1. **Mehran Aalami**, U. J. S. Prasada Rao, and K. Leelavathi, "Studies on the enzyme activities of some varieties of Indian durum wheat". Presented in 5<sup>th</sup> International Food Convention, IFCON 2003, CFTRI, Mysore, 5-8 December, 2003.
2. **Mehran Aalami** and K. Leelavathi, "Spaghetti Making Quality of Indian Durum Wheat". 16<sup>th</sup> Indian Convention of Food Scientists & Technologists (ICFOST), 9-10 December, 2004. CFTRI, Mysore.
3. Sadeghi Mahoonak A.R., **Mehran Aalami**, K. Leelavathi, and Bhagya Swami Lingappa, "Effect of Incorporation of Mustard Protein Isolate on the Quality Characteristics of Spaghetti". 16<sup>th</sup> Indian Convention of Food Scientists & Technologists (ICFOST), 9-10 December, 2004. CFTRI, Mysore.
4. **Mehran Aalami**, Prasada Rao, U. J. S. and Leelavathi, K. "A Protein Marker to Differentiate Good and Poor Indian Durum Wheat Varieties for Spaghetti Making Quality". Colloquim on Novel Proteins in Nutrition and Health. March 22<sup>nd</sup> 2005. CFTRI, Mysore.
5. **Mehran Aalami**, K. Leelavathi, "Effect of High and Low Drying Temperature on Cooking Quality, Starch Properties and Microstructure of Spaghetti". 37<sup>th</sup> Annual Conference of Nutrition Society of India. 18-19 November 2005. Hyderabad.
6. **Mehran Aalami**, K. Leelavathi, and U. J. S. Prasada Rao, "Physico-Chemical and Rheological Characteristics of Indian Durum Wheat: Relationship to Spaghetti Making Quality". 17<sup>th</sup> Indian Convention of Food Scientists & Technologists (ICFOST), 9-10 December, 2005. Bangalore.