INFLUENCE OF *TRIBOLIUM CONFUSUM* DEVELOPMENT ON SELECTED PHYSICAL PROPERTIES OF SEMOLINA

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ABSTRACT

A total of 30 adult Tribolium confusum *were captured from storage semolina silos and placed in 1 kg of fresh semolina. During 2 months of storage in the laboratory, impact of presence and development of these insects on selected properties of semolina were evaluated. Obtained results showed that* T. confusum *development caused an increasing of acidity, ash content, burn color and damaged fractions. Moreover,* T. confusum *presence leads to a loss of gluten properties, semolina water-binding capacity, and an increase in hardness and adhesiveness of elaborate semolina dough. Microbiological evolution shows a constant increase in bacterial load during storage period. This bacterial level remained below the safety limit during the storage period.*

PRACTICAL APPLICATIONS

For pasta and couscous industry, pest could be a major danger during storage of raw materials (semolina, wheat flour, maize flour, etc.). *Tribolium confusum* is one of the major insect present in durum wheat flour and its derivatives. This insect could cause great damage on the nutritional, hygienic, technological and sensorial qualities of this raw material. Evaluation of presence and development of insects, and especially *T. confusum* on selected properties of stored semolina could help pasta and couscous industries to

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determine the critical limits of identified critical control points in the semolina storage step.

KEYWORDS

HACCP, physicochemical and technological properties, semolina, *Tribulium confusum*

INTRODUCTION

Cereal composition is always a key factor in the quality and safety of different human foods. For example, pasta and couscous are cereal-based products largely consumed everywhere in the world. The main steps in the pasta and couscous production chains are: wheat (durum) harvesting, transportation, storage, industrial processing and handling by the consumer. Every step must be covered by specific standards to ensure a high level of food safety and quality. Therefore, it is important to establish a control method such as Hazard Analysis Critical Control Point (HACCP) or International Organization for Standardization (ISO) 22000 to ensure food safety and quality. For pasta and couscous industry, in addition to biological, chemical and physical dangers, the pest could be the major danger during storage of raw materials (semolina, wheat flour, maize flour, etc.). Pest in a pasta and couscous factory could be insects (e.g., *Tribolium confusum*) and/or rodents (e.g., mouse). *T. confusum* is one of the major insect present in durum wheat flour and its derivatives. This insect could cause a great damage on the nutritional, hygienic, technological and sensorial qualities of this raw material (Godon and Popineau 1984). In addition to product deterioration, the presence of insect and pest impurities could lead to a microbiological contamination sometimes too dangerous for human health. Evaluation of this damage could be of interest for critical control points' (CCPs) identification and their critical level. Usually, critical limits of CCPs could be based on current and specific legislation or on published scientific studies. Evaluation of presence and development of insects, and especially *T. confusum* on selected properties of stored semolina could help pasta and couscous industries to determine the critical limits of identified CCP in the semolina storage step.

During recent years, several studies have focused on the development, the distribution and the spatiotemporal dynamics of stored-product insects in various types of flour (Mestres *et al.* 1997; Terzia *et al.* 2003; Morimoto *et al.* 2007). Recently, Trematerra *et al.* (2006) studied the spatiotemporal analysis of trap catches of *T. confusum* du Val in a semolina mill, with comparison with

female and male distributions. Olsson *et al.* (2006) studied the behavior of *T. confusum* and the pheromone production location.

From a technological point of view, several studies were devoted to semolina properties during storage and processing. Hébrard *et al.* (2003) studied the influence of particle size and temperature on the hydration properties of semolina. Erbas *et al.* (2005) studied moisture adsorption behavior of semolina and farina. Azizi and Rao (2005) studied the effect of surfactant gels' storage on the bread-making quality of wheat flour.

Few scientific studies have been devoted to the impact of insects' development on physicochemical, nutritional and hygienic properties of wheat flour and semolina during storage.

In the present paper, impact of *T. confusum* presence and development on selected properties of stored semolina was evaluated.

MATERIALS AND METHODS

Superior-quality industrial semolina (*Triticum durum*, 3SSE type and granulometry <400 μm) samples were used in the present experiments. These samples were obtained from the Tunisian factory of food pasta (STPA: Société Tunisienne des Pâtes Alimentaires) in Sfax, Tunisia.

A sample of *T. confusum* was collected from stored semolina (Couscousserie Du Sud, Sfax, Tunisia) using insect trap catches. Approximately 30 adult *T. confusum* were placed in 1 kg of semolina and kept for 2 months in a climate chamber with light–dark cycle, temperature at 25 $\pm$ 1C and a relative humidity at least at 45%. A semolina sample was taken every 10 days to evaluate the number of insect impurities and the influence of *T. confusum* development on selected properties (nutritional, physicochemical and microbiological) of stored semolina.

Physicochemical Analysis

Water content was determined according to NT 51.20 (1989). Dry gluten, wet gluten and gluten index were determined using a glutomatic system (NT. 51.13 1994). Recuperation of insoluble protein fraction (gluten) from semolina was carried out according to the procedure described by Godon and Popineau (1984). Fat content was calculated according to NF V-03-713 (1996). Ash content in the semolina samples were calculated according to NT 51.34 (1996). The content of starch in semolina was determined using enzymatic method as described by Ellouze-Gorbel (1996).

Specific minerals (K, Mg, Ca and Na) present in the semolina were analyzed by a flame spectrophotometer Hitachi Z-6100 (Hitachi instruments

Engineering Co, Ibaraki, Japan), according to NF 09.09 (French Association for Normalisation, NF 51.34 1996).

Soluble, insoluble and total dietary fiber contents were determined according to Englyst *et al.*'s (1991) procedure. After an acid hydrolysis, the residues are filtered and washed with distilled water. The residues are then washed with 95% ethanol, followed up with a second washing with acetone, then dried and weighed.

Water activity measurements were realized at 25C using a Novasina aW apparatus (SPRINT TH-500, Novasina, France).

Fatty acid content was measured according to ISO 7305 (1998) standard. Obtained results were converted to an equivalent sulphuric acid concentration.

Water-binding capacity (WBC) of semolina samples was measured according to the procedure described by Mestres *et al.* (1997).

Gruel contents correspond to the quantity of powder released by the degradation of semolina seeds. Gruel contents in the semolina samples were quantified using a successive sifting. Rate of gruel was expressed as the difference between initial and final rate of refusal particle percentage in the lower sieve.

Methods of Insect Impurities Counting: Filth Test

The counting of insect impurities in semolina samples was realized according to (ISO 1150 1994). An acidic hydrolysis of semolina particles was carried out after dilution of 50 g of semolina in 300 mL of Milli-Q water and 20 mL of hydrochloric acid. After half an hour of boiling with agitation, the solution was kept in a Becker covered with a protection film. A dens-metric separation of resistant fragments to the hydrolysis was realized. After this decantation, the resistant fragment was concentrated by filtration. Fifty milliliters of ethanol (50%) was added to the solution. After stabilization, insect impurities go up to the interface between the aqueous phase and the ethanol. Contents of the bulbs are filtered using a vacuum filtration set (Büchner). The filter was collected in a petri dish coated by glycerol-coated alcohol. The insect impurities present in the semolina samples were identified and counted using an optical microscope.

Color Measurements

Semolina color was measured with a Minolta colorimeter (Minolta, Tokyo, Japan). Triplicate measurements of L^*, a^* and b^* were done. Measurements as L^*, a^* and b^* values were standardized with a white color standard, where L^* is the brightness coefficient from dark (–) to bright (+), a^* the coefficient from green (–) to red (+), and b^* the coefficient from blue (–) to yellow (+). Results relative to L^* were presented as burn index ($100 - L^*$).

Textural Analysis

For each semolina sample, 100 g was hand-mixed with 25 mL Milli-Q water to obtain a homogenous mixture. A texture procedure analysis test was performed using a texture analyzer (texture analyzer: Lloyd Instruments, England) equipped with a 1,000 (N) load cell and 0.05 (N) detection range.

A sample of hydrated semolina was transferred into a molded Nalgene polypropylene tube (5 cm height) that was placed in a fixture to hold it in place under the texture analyzer. An acrylic cylindrical probe was used to compress the semolina sample by 50% of its original height (40 mm) at a speed of 60 mm/min.

Microbiological Analysis

A 25 g semolina sample was weighed into a glass beaker (500 mL) and mixed with 225 mL of a sterile peptone–water solution (0.1%) using a magnetic stirrer for 20 min to obtain a homogenized sample (Jay 1986).

Aerobic plate count was done using the aerobic spread plate count method described by Jay (1986). Aerobic plate count agar (PCA; Merck, Darmstadt, Germany) was used for the analysis. Dilutions of the sample were prepared, and an amount of 0.2 mL from every dilution was transferred into a corresponding labeled petri dish and spread plated over the agar surface. Inoculated PCA plates were incubated at 35C for 24–72 h. The plates with less than 300 colony-forming units (cfus) were counted, and the average value was taken after a duplicate count. The number of cfus was multiplied by the dilution factor and divided by the inoculation amount in order to determine the cfus per gram of semolina.

Statistical Analysis

Triplicate analysis (physicochemical, insect impurities counting, color parameters, textural and microbiological analysis) were performed on each replicate of stored semolina samples. Values of different tests were expressed as the mean $\pm$ SD ($x \pm$ SD). SPSS (Statistical Package for the Social Science, SPSS Inc., Chicago, IL) packet program for Windows was used for the statistical analysis. Significant differences between mean ($P < 0.05$) were determined by using a one-way analysis of variance (Duncan's test).

RESULTS AND DISCUSSION

Physicochemical Characteristics of Semolina and Infestation Risk

Physicochemical characteristics of semolina samples used in this study are presented in Table 1. Andrieu *et al.* (1986) reported that starch, protein and lipid

TABLE 1.
PHYSICOCHEMICAL COMPOSITION OF SEMOLINA

Composition (% of dry matter)	Mean ± SD
Water content	14.00 ± 0.09
Dry gluten	12.90 ± 0.11
Starch	55.64 ± 0.90
Fibers soluble	2.24 ± 0.05
Fibers insoluble	3.44 ± 0.04
Fatty acidity (g of sulphuric acid/100 g of dry matter)	0.03 ± 0.01
Fat	1.69 ± 0.05
Ash	0.95 ± 0.02
K (mg/100 g of dry matter)	1,056.00 ± 0.91
Na (mg/100 g of dry matter)	111.11 ± 0.55
Ca (mg/100 g of dry matter)	1,477.00 ± 1.22
Mg (mg/100 g of dry matter)	375.16 ± 0.55
pH	6.05 ± 0.11
aW	0.56 ± 0.02

of drum wheat semolina were 70, 14.5 and 2 g/100 g, respectively. Hébrard *et al.* (2003) reported that starch, fat and ash content of semolina were 86.1, 1.4 and 1.2%, respectively. Erbas *et al.* (2005) reported that starch, crude fiber and ash content of semolina were 73.64, 7.05 and 0.772%, respectively. These results are in concordance with values presented in Table 1. Table 1 shows, too, a low water content and a low water activity value, which suppose that semolina infestation risk was very low. However, stored product insects have developed specific requirements concerning the constituents of their diets. Because of low water content of dried food products, these insects have characteristically adapted to require less water in their diets (Levinson and Levinson 1978). In a study on larval development, semolina and soybean flour were both excellent diets for stored-product insects, resulting in faster development than when wheat alone was used as a diet (Locatelli and Biglia 1995). Indeed, semolina and soybean flour contain the necessary fatty acids, steroids and vitamins. Locatelli and Biglia (1995) also tested different semi-manufactured products containing different mixtures of the individual ingredients. These authors conclude that the infestation risk is higher for semi-manufactured products since they provide all nutrients required for a successful larval development for several insect pests. Table 1 shows that semolina samples used in this study contain the major elements necessary to insect development.

T. Confusum Impurities Changes

Figure 1 shows insect impurities evolution versus storage time, and Fig. 2 shows an example of photos of impurities observed in stored semolina.

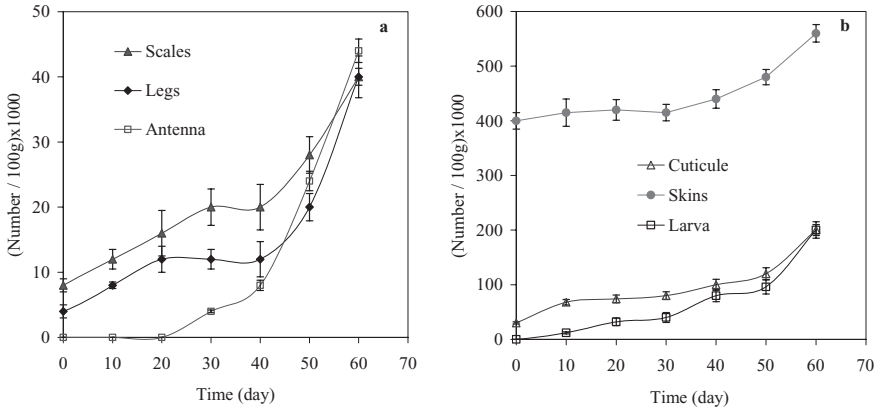

FIG. 1. INSECTS' IMPURITIES IN SEMOLINA VERSUS STORAGE TIME
(a) Scales, legs and antenna evolutions; and (b) cuticle, skins and larva evolutions.

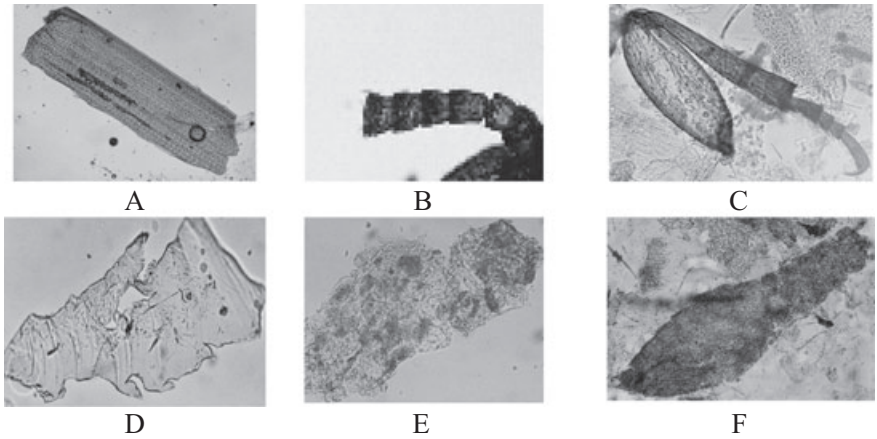

FIG. 2. AN EXAMPLE OF INSECT IMPURITIES PHOTOS OBSERVED IN STORED SEMOLINA
(A) Scales; (B) antenna; (C) legs; (D) cuticle; (E) skins; and (F) larva.

Figure 1 shows that the insects' impurities' number increases significantly from the first month of storage. Indeed, Fig. 1 highlights a significant increase in rates of scales (Fig. 2A), legs (Fig. 2C), antennas (Fig. 2B) and cuticule (Fig. 2D) after 20–30 days. These insects' impurities increasing could be explained by *T. confusum* number increase, corpse degradation and insects' biological activity (e.g., skin and body change). Indeed, Smith *et al.* (1971)

reported that hard wheat flour infested by *T. confusum* showed no changes in number of adult insects at the end of 1 month, but rapidly increased their number during the 2- and 3-month infestation periods.

Evolution of Semolina Characteristics during Storage

Physicochemical Parameters Evolution. Figure 3 shows evolution of semolina water content and water activity versus storage time. This figure shows low initial values of humidity and water activity (14% and 0.56, respectively). This value of semolina humidity conformed with Codex Alimentarius (Codes Stan 178-1991) recommendation concerning humidity of semolina used for pasta and couscous industries. Figure 3 highlights a consistent decrease in water activity and water content. This water content and water activity chute could be explained by both water exchange between semolina and the ambient air and activity of insects present in the semolina. It is well known that although semolina has a low rate of water (content and activity), *T. confusum* could develop, thanks to their adaptation to require less water in their diets (Levinson and Levinson 1978). Moreover, insects' development increased respiration in the semolina (hot spots), and this, associated with the metabolic activity of the pests themselves, promotes evolution of heat and moisture content in the material.

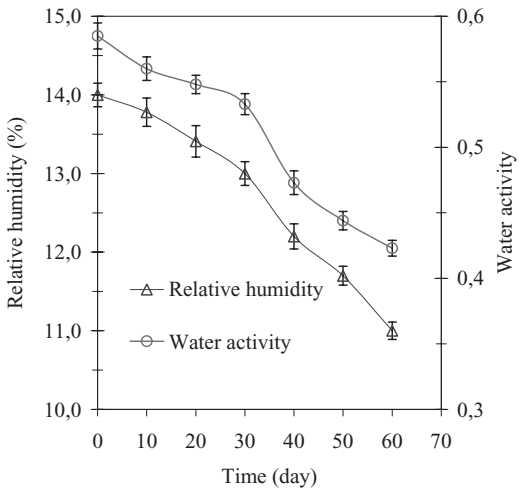

FIG. 3. SEMOLINA RELATIVE HUMIDITY AND SEMOLINA WATER ACTIVITY VERSUS STORAGE TIME

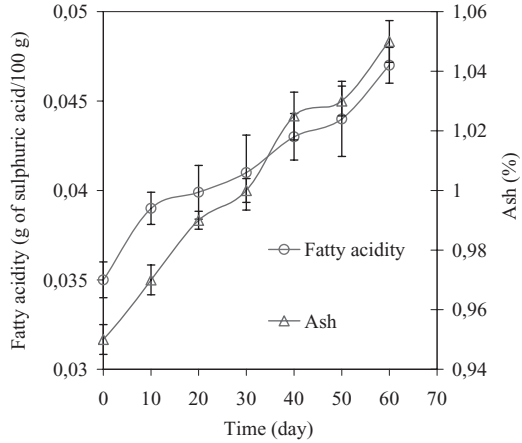

FIG. 4. ASH CONTENTS AND FATTY ACIDITY DURING STORAGE OF SEMOLINA

Fatty acidity and ash rate evolution versus storage time were presented in Fig. 4. Although Fig. 4 clearly shows an increase in semolina acidity and ash rate, these values remained to conform to the reglementation even after 60 days. Indeed, Codex Alimentarius recommend (Codex STAN 178-1991) an ash rate for semolina less than 1.2% of dry matter. After 60 days, fatty acidity in stored semolina samples was less than 0.05 g H_2SO_4/100 g, a value recommended by French normalization (NF V50-001 1992).

Phothist and Charoennein (2007) reported that the increase of fatty acidity in flours and semolina is due primarily to the fermentation activity of present microorganisms. This activity strongly depends on the temperature and causes the release of the organic acids in the samples. Moreover, the biological activity of insects in the semolina is characterized on the one hand by the release of uric acid excrements, and on the other hand, by a local heating. This local heating accelerates residual fermentation. Degradation of fat content present in the semolina also takes part in fatty acid increase by the release of free fatty acids responsible for rancid smell. In addition to fatty acid increase, a light acidification of semolina samples was recorded. Indeed, pH decreased from 6.05 for the initial semolina samples to 5.81 for semolina samples after storage during 2 months.

Figure 4 shows an increase in ash rate during storage period. This increase is more significant after the first 20 days. Degradation of biomaterial such as insects' corpses, and, more particularly, cuticles (substances rich in chitin), are at the origin of ash rate increase.

Figure 5 shows color parameters during storage time. Burn index was expressed as 100 – L^*, which represents sample lightness evolution. Figures 5

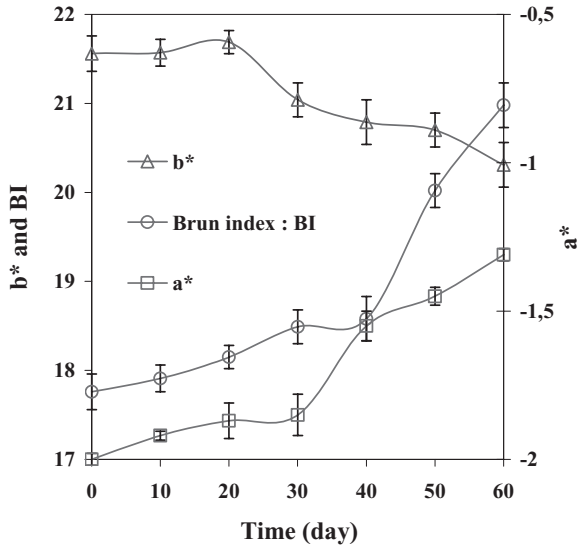

FIG. 5. COLOR PARAMETERS DURING STORAGE OF SEMOLINA
BI, burn index (100 – L^*).

shows that burn index and a^* increases during storage time. However, there was a small increase in b^* parameter during the first 20 days, then a consistent decrease from day 20 to 60. These evolutions of color parameters could be explained by the release of insects' fragment following biological activity and corpse's degradation. Indeed, *T. confusum* was characterized by their red-brown color.

Technological Parameters Evolution. Gruel content evolution, corresponding to the amount of powder released by semolina seeds degradation, is illustrated in Fig. 6. This degradation could be the result of insects' activity (remains of their food and their waste) or molds' development, which is characterized by the release of dusty fine waste. Figure 6 highlights an increase in the gruel rate during two storage months (from 8 to 12%). Moreover, after the 40th day, this rate increased significantly. This date corresponded to a significant increase in insect impurities (Fig. 1).

WBC is one of the functional properties of proteins most required in agroalimentary industry. For wheat-derivative products, this capacity depends primarily on the nature and the space structure of gluten proteins (presence of disulfide bonds). Figure 6 presents variation of dry gluten rate and WBC of semolina samples versus storage time. A steady reduction in dry gluten level and WBC was observed. This loss of WBC is due primarily to denaturation and

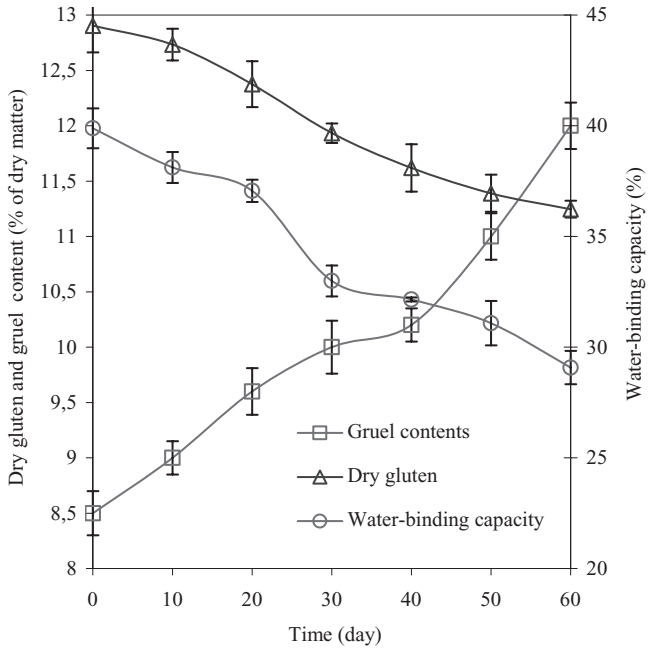

FIG. 6. DRY GLUTEN RATE, GRUEL CONTENTS AND WATER-BINDING CAPACITY DURING SEMOLINA STORAGE

partial hydrolysis of gluten under the effect of acidity variation (uric acid and organics acid relegation) and temperature change (insects, molds, bacteria and enzymes activities). Indeed, insects' development causes an increase on local temperature (hot spots), CO_2 and moisture content (respiration phenomena), which accelerate molds and bacteria development in the semolina samples, and production of acidic compounds. These activities and environment change induce a change on proteins nature and space structure that lead to a reduction of semolina WBC. In addition to WBC, the reduction of insects' and microorganisms' activities leads to change on texture characteristics of dough semolina samples. Table 2 presents hardness, springiness and adhesiveness evolution during storage time. Table 2 shows a significant difference ($P < 0.05$) between initial hardness value and value obtained after 30 and 60 days of storage. A significant difference ($P < 0.05$) was observed between initial and final adhesiveness value. However, no significant difference was observed between initial, after 30 days and final springiness value. These textural changes could be explained by WBC reduction and modification of hydrophobic interactions between proteins, lipids, starch and soluble compounds present in semolina.

TABLE 2.
INSTRUMENTAL TEXTURAL PARAMETERS OF SEMOLINA DURING STORAGE

Instrumental textural parameters	Initial	After 30 days	After 60 days
Hardness	4.40 ± 0.09[a]	5.58 ± 0.07[b]	8.97 ± 0.10[c]
Springiness	14.33 ± 0.12[a]	14.33 ± 0.17[a]	15.04 ± 0.11[a]
Adhesiveness	2.99 ± 0.03[a]	7.2 ± 0.02[b]	7.66 ± 0.08[b]

Different letters in the same line indicate significant differences ($P < 0.05$).

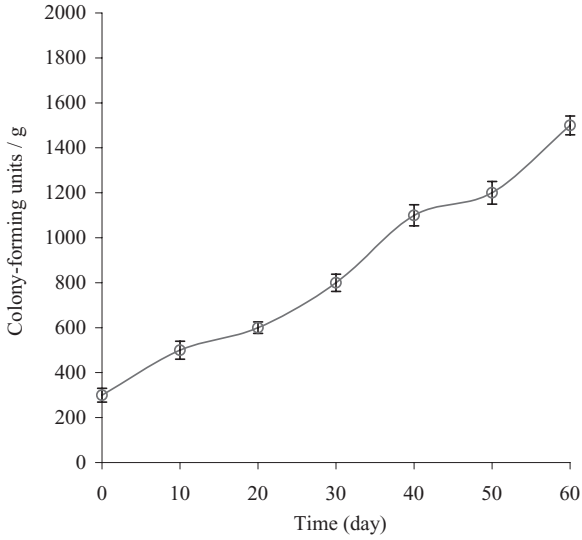

FIG. 7. TOTAL AEROBIC BACTERIA NUMBER DURING SEMOLINA STORAGE

Microbiological Evolution. Semolina is generally regarded as a microbiologically safe product, as it has relatively low water activity (i.e., <0.50). Figure 7 shows that under the study conditions, semolina had low levels of microfloral infection. Storage fungi such as *Eurotium* spp. and *Penicillium* were virtually absent during 2 months of storage. A steady increase in bacterial infection was observed during 2 months of storage, although bacterial populations did not increase at low moisture content. This increase was attributed to insect contamination and change in insect development environment (self-heating, CO_2 production, local moisture change, etc.). This bacterial level remained below the safety limit (Sauer *et al.* 1992).

Summary and Conclusion

HACCP is utilized worldwide as the most important tool for guaranteeing the safety of consumable foods. Establishment of critical limit and tolerances for CCPs and minimum hygienic requirement for operational preestablished programs must be based on regional or/and international legislation and scientific advice. European rules related to hygiene in handling with food demands to avoid any negative affecting of food. Pest and their excrements affect food in this way. Main hazards could be occurred by pest infestation in the semolina industry: bacteria transmission (sanitary hazard), product damage (technological damage), negative effect on taste, smell and appearance modification (organoleptic damage). These hazards and damages could influence quality of the final product, such as bread, pasta and couscous.

From a technological point of view, this study shows that the development of pest, essentially *T. confusum,* could lead to gruel production and a loss of gluten properties and WBC of semolina. This latter property is very important for the final products' texture as pasta or couscous. Indeed, when pasta is cooked al dente (al dente refers to the desired texture of cooked pasta in Italian cooking), there should be a slight resistance in the center when chewed. This resistance depends on the capacity of pasta to absorb water during cooking. Durum semolina is also used to manufacture couscous, which is a staple for many North African countries, and, as it is so versatile, it is gaining in popularity in Europe and North America. Organoleptic and texture property of couscous depend essentially on the capacity of semolina to absorb water vapor during cooking and sauce after cooking. It is evident that the capacity of semolina to absorb water before and after cooking is a major characteristic of this raw material. Pest infestation of this raw material could lead to a change of the final product quality.

From organoleptic point of view, the development of *T. confusum* in semolina during storage leads to an increase in acidity, ash content, burn color, and damaged fractions. These organoleptic changes could affect the quality of the final products (taste, color, texture, etc.). Color of pasta and couscous is a major quality parameter, which depends on Maillards reaction during drying process. Initial semolina chemical (sugar and protein) and color (clarity) characteristics are of great importance to ensure a stable and required color of pasta and couscous.

From sanitary point of view, this study shows that in spite of the presence and development of *T. confusum*, microbiological contamination of stored semolina remained below the safety limit. This sanitary insurance is due to low moisture content and water activity of semolina.

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