ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES AND MICROBIAL CONTAMINATIONS OF LOCAL AND IMPORTED COUSCOUS

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Abstract

Limited studies have been carried out to investigate the physicochemical properties and microbial contaminations of couscous in Libya. The purposes of the investigation were to determine the physicochemical properties and microbial contaminations of locally manufactured couscous, traditional couscous and imported couscous. Five samples of local couscous were monthly collected from different factories. Additionally, twenty fresh samples and twenty sunlight-dried samples were collected from different regions in Tripoli, and twelve samples of imported couscous were collected too. The results of protein content in traditional dried, local and imported couscous were 11.99, 10.81, and 12.04%, respectively. Traditional wet and dried couscous recorded the highest ash content. Fiber content and acidity for all samples were in the recommended limits. The amount of lead and cadmium were in the recommended limit (0.0003 and 0.012 mg/kg, respectively). The results showed that local couscous had the highest heterotrophic bacteria, 3.03 CFU/g, then comes the traditional dried couscous 2.92 CFU/g, and finally comes the traditional wet couscous 2.88 CFU/g. Yeasts and molds found in local and traditional dried couscous exceeded the maximum limit indicated by WHO (2.99-3.18 CFU/g). The outcomes presented a greater content of molds in fine semolina compared to coarse semolina. Most of the measured properties of the studied samples are in the recommended limits, however, more heather condition are needed during couscous production.

Keywords: Traditional Couscous; Local Couscous; Imported Couscous; Physicochemical Properties; Microbial Contamination.

Introduction

Couscous is one of the oldest cereal foods of the Arabic Maghreb countries, which is produced from durum wheat semolina. It ranks the second after pasta in popularity and consumption around the world. It is generally produced in North Africa and it is becoming more widespread in some European countries, mainly in the Mediterranean region. This tendency is certainly related to the increasing interest to the so-called *ethnic foods* and the increment of the Arabian-origin population living in Europe (Hammami and Sissons, 2020).

Durum wheat semolina is the main raw material for couscous production although different cereals, like maize, sorghum and millet are utilized particularly in West and sub-Saharan Africa for couscous preparation (Schoenlechner, 2016). Couscous is conventionally prepared by hand mixing of semolina and salted water until granules are formed. The lumps are initially sieved to attain a uniform size, and then steamed, sun-dried and stored. The drying stage is strictly linked to the environment conditions, specifically for the homemade couscous (Hammami and Sissons, 2020).

The industrialized production of couscous began in ‘70s (Debbouz and Donnelly, 1996). The utilized process is the same as that used by home-made producers. After mixing of semolina with salted water are carried out, the aggregates formation is accomplished in special rotary sieves, which also allow the granules to be separated based on size and it removes the non-hydrated semolina fractions. The produced couscous granules are then located on a belt and initially cooked by steaming. This process plays a significant role in measuring the quality of couscous. Indeed, the influences on physical and cooking characteristics of couscous quality are basically related to the steaming temperature and time, in addition to the steam diffusion inside the mass (D’egidio and Pagani, 2010). The main advantages of pre-cooking is the reduction of cooking period. Drying step is usually performed in short pasta dryers, followed by cooling and sieving (Quaglia, 1988). It was found that the quality of couscous differs with the alteration in the hydration degree, regardless the used semolina. In fact, it seems that the increase in hydration rate leads to couscous elevation which results in greater granulometry and better uniform product although the color is degraded. In terms of cooking quality, irrespective to the negative effects of hydration degree on producing a sticky couscous, its elevation has positive effects on swelling ability of couscous and solubility index of starch (Lefkir *et al*., 2017).

Even though the couscous consumption is increasing, there are few studies have investigated the role of process parameters and the raw materials on its quality. In terms of couscous quality, uniform granules size, pleasing color and acceptable flavor are the most measuring parameters for dry aggregates quality (Debbouz and Donnelly, 1996; Ounane *et al*., 2006). Protein quality and quantity could affect couscous quality. Some studies stated that the reduction in stickiness associates with the increment in protein content (Debbouz *et al*., 1994). Whereas, other studies indicated that there is no significant correlation between couscous properties and the quantity and quality of semolina protein or gluten (Ounane *et al*., 2006). In addition, the amount of starch damage in semolina might play a role in regulating the water absorption in both cold and hot media (Debbouz *etal*., 1994; Pagani *et al*., 2009). An investigation established the significance of lipid fraction on couscous properties (Ounane *et al*., 2006). For example, polar free lipid content of semolina was certainly connected to firmness of the product; however, contents of free lipid and non-polar lipid negatively correlated with aggregate swelling. Another role of lipid is its association with the rancidity phenomena during storage (Pagani *et al*., 2009).

The nutritional value of couscous is considered good, specifically for couscous that prepared from whole-wheat semolina. It was found that one third of a cup of dried couscous produced from whole-wheat semolina has 8g of protein, 44g of carbohydrates, 1g of fat, and 7g of dietary fiber and about 220 calories (NHDHS-DPHS, 2009). Moreover, it was established that 100g of cooked couscous contains 72.57g of water, 3.79g of protein, 23.22g of carbohydrates, 0.10g of total sugars, 0.16g of fat, 0.029 of saturated fat, 1.4g of fiber, 0.26g of ash, 8mg of magnesium, riboflavin (0.027mg), thiamine (0.063mg) and about 112 calories (Coskun, 2013). A study was performed by Filon et al. (2013) indicated that cadmium and lead contamination of some couscous samples did not exceed the recommended limits.

Microbial contamination of grains and their products should be taken into consideration; since this type of food is favored by many types of mold that could produce toxins. A study was conducted on microbial contamination in wheat grains showed that the majority of contamination with microorganisms and fungi was in the outer layers of wheat grains (Laca, 2006). Another study was conducted in Nigeria on the contamination of stored food with fungi and its toxins, indicated that the contamination is due to environmental conditions (Atanda et al., 2013). The results of an examination of cooked and dried barley semolina revealed that the total numbers of aerobic bacteria and molds in the couscous were $7 X 10^4$ و $7.5 X 10^1$ (Yamlahi et al., 2014). It was pointed out that 27 types of molds were isolated from different cereal products, seven of which were mycotoxins producer (Doolotkeldieva, 2010).

Libya is one of the countries that regularly consume couscous, which is mostly either homemade or in the local factories. It is also imported from other countries like Tunisia, Algeria, Italy and Turkey. However, rare studies have been performed to explore their physicochemical properties and microbial contaminations. Accordingly, the objectives of the current study is to measure the physicochemical properties and determine the chemical contamination of the couscous produced locally in Tripoli factories using durum wheat semolina, the homemade couscous produced in different areas in Tripoli and the imported ones found in some supermarkets in Tripoli.

Materials and Methods

Materials

In this study, five samples of locally produced couscous were monthly collected at a two-hour interval during the production day, and then these samples were mixed to have a representative sample for the production day. In addition, forty homemade couscous samples including 20 fresh samples and 20 dried direct sunlight samples were collected from some regions in Tripoli. Moreover, 12 samples of different imported couscous brands which weighed 1 kg each were purchased, and then they were given the symbols of A, B, C, D, E, F, G, H and I. the imported ones were produced in Tunisia, Algeria, Italy and Turkey. Besides, fine and coarser semolina samples were monthly collected throughout the manufacture day at continual intervals (each two hours), followed by mixing these samples to form a typical sample for every month. Fine and coarser were utilized for only microbiological analysis.

Methods

Determination of Physicochemical Properties

The physicochemical properties of the samples were monthly measured according to the method approved by AACC (1976). These properties included moisture, ash and crude fibers. Protein was determined according to AOAC (2005). As for the determination of acidity, it was estimated as sulfuric acid (LNCSM, 1982). Lead and cadmium was measured following the method of British standard (2003).

Microbial Tests

The samples were prepared under sterile conditions and microbiological tests were monthly conducted (Midura and Bryant, 2001). The numbers of heterotrophic bacteria were estimated using the plate count agar (Morton, 2001). Determination of coliform bacteria was performed using violet red bile agar (Kornacki and Johnson, 2001) while, estimation of the total count of fungi was accomplished by using potato dextrose agar (Beuchat and Cousin, 2001). Isolation and purification of the dominant mold colonies was done by microscopy following the method of Singh et al. (1991).

Statistical Analysis

Statistical analysis was accomplished by using Complete Random Design (CDR). To find out the significant differences between the means at 1% probability level, Duncan's test was conducted by using the Statistical Analysis System (SAS, 1998) program.

Results and Discussion

The Physicochemical Properties

Table (1) shows the general average moisture content of traditional dried, local and imported couscous that were 10.86, 9.35 and 10.5%, respectively. These results comply with Libyan standardization for couscous (LNCSM, 2009). It can be noticed that the moisture content range of the dried traditional couscous was 8.54 – 13.17%. This fluctuation in the moisture content in this type of couscous was expected due to its dependence on sunlight for drying compared to the controlled drying conditions in couscous factories. As for the traditional wet couscous, high moisture levels were detected up to 31.97%, because of hydration process during granulation. Also Lefkir et al. (2017) reported that traditional couscous has different hydration rates namely 34%, 36% and 38%. The high moisture content leads to the rapid development of chemical and microbial decompositions. Accordingly, this type of couscous is preserved under low temperatures such as refrigeration or freezing. It is worth mentioning that Libyan standardization for couscous did not refer to this type of couscous. The results showed that there were significant differences in the moisture content among all types of couscous ($p \leq 0.01$).

The average results of protein content in traditional dried, local and imported couscous were 11.99, 10.81, and 12.04%, respectively, as shown in Table (1); these results are consistent with the limits of Libyan standardization for couscous. While the results of a study conducted by USDA (2010) on a locally manufactured couscous do not agree with the current results. The data pointed out that there was a fluctuation in protein content in the studied samples; this fluctuation might be because of the variation in the protein of the used semolina, or due to protein loss that occurs during the grinding and extraction phase of the used hard wheat. This could affect the quality during cooking process couscous in terms of water absorption and couscous stickiness during the evaporation phase. Findings also indicated that there is a significant difference between the locally manufactured couscous sample and the rest of the samples ($p \geq 0.01$). It was also noted that the average protein content in wet couscous was 12.03%, which is comparable to that of imported couscous; the reason could be due to the additional protein from wheat flour that is used during making couscous granules of the traditional wet couscous.

The average ash content was 2.48, 0.85 and 1.07%, in the traditional dried, locally manufactured and imported couscous, respectively, as shown in Table (1). The ash content in the locally manufactured and imported couscous complies with the limits of Libyan standardization (LNCSM, 2009). It also agrees with the results of a study conducted by USDA, (2010). As for the traditional wet and dried couscous, ash content was high (1.98 and 2.48%, respectively). The high ash content in these two types of couscous might be caused by the drying method used; as the dried couscous is exposed to air pollution during the solar drying stage, as well as due to the addition of wheat flour and a small amount of sodium chloride during preparation process of these types of couscous. Kramer and Twigg (1970) indicated that high ash content in semolina caused a decrease in the quality of the couscous product represented in obtaining an undesirable color. The reasons for the high percentage of ash in some samples of imported couscous, 0.81 to 1.32%, could be as a result of the contamination caused by the equipment or the environmental conditions during processing phase. Significant differences were found in the ash content ($p \geq 0.01$) among all types of couscous.

Table (1) shows the average percentage of acidity and fiber in the traditional dried, the locally manufactured and the imported couscous, which was 0.006, 0.003, 0.007% and 1.89, 0.84%, 0.88% respectively. These two tests are considered important for quality indicator. Acidity above the permissible limits of 0.1% causes unwanted clumping in the couscous and has adverse effects on the characteristics of couscous (Sramkova et al. 2009). Also, the high fiber content leads to a low protein quality in the couscous (Ibid). These results are a significant indicator of the quality of the studied couscous samples and are in agreement with the results of USDA, (2010). The proximate composition and the nutritional value of couscous are evidently dependent on the raw materials used and on the extraction rate of the semolina. A study indicated that the nutritional value of couscous prepared from durum wheat semolina were 8.3 g/100g moisture content, 12.0 g/100g protein content, 1.5 g/100g lipid content, 70.2 g/100g carbohydrates content and3.8 g/100g dietary fiber content (Carcea et al., 2017).

Table (1) presented the average amount of lead and cadmium, which was less than 0.012 and 0.0003 mg/kg, respectively, for all the studied samples. These results agree with the standard requirements of the Libyan standardization for couscous (LNCSM, 2009) that states the amount of lead and cadmium should not exceed 0.2 and 0.1 mg/kg for couscous, respectively. Furthermore, these results are consistent with another study by Filon et al. (2013) where the content of lead and cadmium in couscous was 0.120 mg/kg and 0.025 mg/kg, respectively. Low levels of these two components are of great health importance. These results indicate that the sources of wheat used were far from chemical and industrial contaminations.

Table (1): Proximate Composition, Dietary Fiber, Heavy Metals and Acidity of Couscous Samples.

Couscous Type		Moisture (%)	Protein (%)	Ash (%)	Dietary Fiber (%)	Acidity
Traditional Wet	Mean	31.97±0.21[a]	12.03±0.17[a]	1.98±0.05[a]	1.03±0.03[a]	0.007±0.00[a]
	Rang	24.80-39.14	9.85-14.21	1.24-2.72	0.79-1.27	0.003-0.01
Traditional Dry	Mean	10.86±0.21[b]	11.99±0.18[a]	2.48±0.05[b]	1.89±0.03[b]	0.006±0.00[a]
	Rang	8.54-13.17	9.78-14.20	1.15-3.81	0.85-2.93	0.002-0.01
Local	Mean	9.35±0.19[c]	10.81±0.15[b]	0.85±0.04[c]	0.84±0.03[c]	0.003±0.00[b]
	Rang	8.82-9.87	10.72-10.90	0.77-0.92	0.80-0.88	0.002-0.004
Imported	Mean	10.5±0.28[b]	12.04±0.22[a]	1.07±0.06[d]	0.88±0.04[d]	0.007±0.00[a]
	Rang	8.35-12.65	11.18-12.89	0.81-1.32	0.83-0.93	0.003-0.01
LNCSM, 2009		Maximum 12.5%	Minimum 10.5%	Maximum 1.1%	-	-

The values are the mean ± standard deviation. Values with the same subscribe letters are not significantly different at p ≥ 0.01. LNCSM = Libyan National Center for Standardization and Metrology.

Microbial Contamination

The results showed an increase in the number of heterotrophic bacteria of the local couscous, followed by the traditional dried couscous and then the traditional wet couscous, while the imported couscous was the least contaminated Table (1). The results showed that there were significant differences between them ($p \leq 0.01$). It is noted that the results of all types of couscous conformed to the maximum limits 5.3 CFU/g and 4.00 CFU/g, which were indicated by Fiche Technique Couscous (2008) and World Food Program Organization (2015), respectively.

The presence of contamination, even in low numbers, might be due to the unfavorable environmental conditions during production. The decrease in the numbers of heterotrophic bacteria is not sufficient evidence of the availability of health conditions for the couscous due to the possibility of the presence of some types of molds that could produce mycotoxins as mentioned by Wong, (2013). It was noted that coliform bacteria was not recorded in the all samples Table (1), and these results are consistent with the limits of Fiche Technique Couscous (2008), which indicated that the number of colon bacteria should not exceed 1.00 CFU/g.

The results revealed that the average number of yeasts and molds found in the local, and traditional dried couscous exceeded the maximum limits (3.00 CFU/g) which were indicated by Fiche Technique Couscous (2008), and that established by World Food Program Organization (2015) (2.00 CFU/g). It can be observed that traditional wet couscous also contained high levels of molds (3.00 CFU/g) whereas the imported couscous had low levels of molds with an average of 2.42 CFU/g Table (2). The results of the statistical analysis indicated that there were significant differences

($p \leq 0.05$) between the imported couscous and the rest of the couscous types. This is consistent with the study conducted by Boutrif and Morse, (1976) in USA, who indicated that couscous prepared in North Africa may be a potential source of mycotoxins producing molds.

Table (2): Heterotrophic Bacteria, Coliform Bacteria, Yeasts and Molds in the Couscous Samples.

Type of couscous	Bactria (CFU/g)			coliform bacteria CFU/g	Molds and yeasts (CFU/g)		
	Mini Limit	Max limit	Average		Mini limit	Max limit	Average
Traditional wet	2.75	3.00	2.88 ± 0.03^{a}	Less than 10	2.80	3.18	2.99 ± 0.07^{a}
Traditional dry	2.79	3.04	2.92 ± 0.03^{b}		2.84	3.36	3.10 ± 0.07^{a}
Local	2.91	3.14	3.03 ± 0.06^{c}		2.95	3.40	3.18 ± 0.14^{a}
Imported	2.24	2.96	2.60 ± 0.03^{d}		1.72	3.12	2.42 ± 0.08^{b}

The values are the mean ± standard deviation. Values with the same subscribe letters are not significantly different at $p \geq 0.05$. Max = maximum limit, Min = minimum limit.

Table (3) shows some types of mold that isolated from the traditionally dried couscous and local and imported couscous. The types of mold that were identified in the samples are among the common molds in foods such as grain products. These molds are widespread in soil, air, and dust and they spoil such types of food and produce mycotoxins.

It was noticed that the isolated species belong to the genus of Aspergillus. Aspergillus flavus was isolated from the imported couscous and it may produce aflatoxin. Aspergillus Niger was also found in the traditionally dried couscous and local couscous. It was reported that some strains of Aspergillus Niger might produce ochratoxin (Doolotkeldieva, 2010). Aspergillus fumigates was also isolated from imported couscous, and this mold might be a human pathogenic species as it produces gliotoxin. In addition, Aspergillusterrus was isolated from imported couscous and could originate from soil and dust, which would cause opportunistic infections to persons who immunocompromized. Alternariaalternata was isolated from traditional dried couscous, and the exposure to this fungus was found to cause asthma symptoms in US residences (Salo et al., 2006). Penicillium can be citrinin-producing while Mucorspp and Rhizopusspp are not mycotoxins, but they can spoil the food (Schuster et al., 2002). The causes of this contamination can be due to wheat products exposure to dust during the manufacturing, drying and packaging processes (Boutrif and Morse, 1976; Wong, 2013).

Table (3): The Isolated Molds in Couscous Samples.

Type of mold	Local couscous	Imported couscous	Traditional dried couscous
Aspergillusflavus	-	+++	-
Aspergillus fumigates	-	+	-
Aspergillusniger	+	-	+++
Aspergillusterrus	-	+	-
Mucorspp	-	+	-
Penicilliumspp	-	+	+++
Rhizopusspp	-	-	-
Alternaria alternate	-	-	+
No growth (-), Light presence (+), Medium presence (++), Intense presence (+++).			

The results exhibited an increase in the number of heterotrophic bacteria for fine semolina compared to rough semolina Table (4) although their results conformed to the limits of microbiological standards for Turkish foods, which indicated that the number of heterotrophic bacteria does not exceed 3 - 4 CFU/g (Turkish Food Codex, 2011). The high number of heterotrophic bacteria can be due to the contamination of durum wheat grains with microorganisms in the farms, during storage before the milling process, or from the equipment during the production process as indicated by Hoseney (1994).

Table (4) demonstrates that the presence of coliform bacteria (CFU/g) was not recorded in all samples of coarse and fine semolina, which are consistent with the limits of microbiological standards for Turkish foods that do not exceed 2-3 CFU/g (Turkish Food Codex, 2011). The results showed a slight increase in the number of yeasts and molds for fine semolina compared to coarse semolina. However, the number of yeasts and molds in both types of semolina agree with the microbiological standards of Turkish foods, which recommend that the number of yeasts and molds does not exceed 3 - 4 CFU/g (Turkish Food Codex, 2011). Moisture content is a critical factor that should be taken into consideration in terms of contamination with microorganisms. Hoseney (1994) stated that moisture should not exceed 14% to avoid contamination and to extend the shelf life of grains and grain products.

Table (4): Heterotrophic Bacteria, Coliform Bacteria, Yeasts and Molds in the Used Wheat Semolina as CFU/g.

Type of Couscous	Heterotrophic Bacteria			Coliform Bacteria	Molds and Yeasts		
	Min limit	**Max limit**	**Mean**		**Min limit**	**Max limit**	**Mean**
Rough semolina	2.66	3.00	2.83±0.04[a]	Less than 10	2.92	3.09	3.00±0.11[a]
Fine semolina	2.68	3.08	2.88±0.05[a]		2.96	3.14	3.05±0.02[a]
The values are the mean ± standard deviation. Values with the same subscribe letters are not significantly different at $p \geq 0.05$. Max = maximum limit, Min = minimum limit.							

Conclusion

It can be concluded that the vast majority of the measured characteristics of the studied samples were in the recommended limits by LNCSM. In addition, the measured heavy metals (lead and cadmium) contents did not exceeded LNCSM recommended limits. Yeasts and molds, which were found in the local and traditional dried couscous exceeded the maximum limit reported by WHO. Thus, we recommend more studies on these type of products in terms of their healthier and functional properties.

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