

SOME NUTRITIONAL AND MICROBIOLOGICAL ASPECTS OF APPLES OF COMMON VARIETIES AVAILABLE FOR HOUSEHOLD CONSUMPTION

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ABSTRACT

Red delicious, Kala kulu and Golden delicious apples taken from different marketing locations and retail stores during minimum availability period were analyzed for nutritional and microbial quality. Twenty one samples were analyzed for proximate composition and 72 samples were examined for surface contaminants using standard techniques. The highest weights of large, medium and small sized apples belonged to Red delicious variety. Peeling reduced both volume and pH of apple juice extracted. The energy, carbohydrates, fat, protein and fiber contents were high in Golden delicious variety while moisture in Kala kulu and the ash contents were high in Red delicious. Bacteriological analysis showed that gram +ive bacteria were the dominant strains associated with apple surface. The most common genus of fungi grown on the surface was *Aspergillus* and *Penicillium*. It was observed that washing with cold running tap water reduced the microbial contamination on apple surface.

Key words: Apple; proximate composition, microbial quality, decontamination.

INTRODUCTION

Pakistan produced 35,1900 tones of apple during 2007 and its cultivation was limited and restricted to upland areas of Balochistan and northern hilly tracts of the Punjab and Khabar Pukhtonkha (Khan *et al.*, 2007). Various varieties of apple which are being grown in Pakistan include *Amri*, *Red delicious*, *Mashhadi*, *Kala kulu* and *Golden delicious* (Abid, 2005). Apple is a highly nutritious fruit containing essential food elements such as carbohydrates, protein, fat and water. Apart from its energy value, apple is a good source of soluble and insoluble fiber (Herforth., 2000). Apples are a widely consumed, rich source of phytochemicals, epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes due to strong antioxidant activity. (Marchand *et al.*, 2000).

Besides nutritional merits, apple may get contaminated during growth, harvest, transportation and further processing & handling with microbes from soil, air, water or animal wastes. Handling in stores and retail markets could also add more microorganisms to the surface of the fresh produce. Janisiewicz *et al.* (1999) demonstrated that fruit flies could transmit *E. coli* to uncontaminated apple wound. Among microbes, *Penicillium expansum*, a fungus is a major causative agent of post harvest decay in apple, since it produces patulin, a mycotoxin known to cause harmful effects in humans (Chen *et al.*, 2004). Washing with water alone reduces the recoverable population of microbes by almost 2 logs from the starting population (Wisniewsky *et al.*, 2000).

In Pakistan, published work on nutritive value and microbial contamination of apple is scanty though it is the most popular fruit and is sold at considerably high price. It was therefore considered necessary to assess the nutritive value of different apple varieties and to examine microbiologically the surface contaminants.

MATERIALS AND METHODS

Nutritional quality of common apple varieties that are available for household consumption was studied. During the minimum availability period (April, May and June), samples of apple were collected from different market locations and retail stores in Faisalabad. These areas were located in Jinnah colony, Gulberg colony, Jail road, Aminpur bazaar, Bhawana bazar, local mendies and shops.

Nutritional analysis: Kala kulu, Red delicious and Golden delicious were the most common and easily available varieties in Faisalabad city. A survey of different markets was done for the purpose of weighing apples of Kala kulu, Red delicious and Golden delicious varieties for grading. For measuring the percentage composition of different apple parts, a total of twenty one samples seven from each variety were randomly selected and cut into flesh, peels and seeds and then weighted. Randomly selected twenty one samples of apples were used for juice extraction. The samples were weighed and then peeled off. Seven samples from each variety were subjected to juice extraction with and without peels and pH values were also determined. The nutrient contents of total twenty one samples of Kala kulu, Red delicious and Golden delicious were determined. Dried samples of apple flesh, peels and seeds were ground. Powdered apple

flesh was used for analyzing protein, fat, carbohydrates and ash using Official Methods of Analysis (AOAC, 1997). While peels were analyzed for fiber content. To calculate the energy of a sample, the percentages of carbohydrates, protein, and fat were multiplied by 4.15, 5.65 and 9.40, respectively and the sum of these values was taken as gross energy (Atwater and Bryant, 1899).

Microbiological analysis: For microbiological examination, samples were collected in polythene bags and stored at 4°C till analysis in the laboratory. For bacterial analysis, standard protocols was used (Awan and Rehman, 2005). For the bacterial isolation, nutrient agar was used as a medium. The apple sample collected from different marketing locations was inoculated on agar medium by wet swabbing. After incubation of samples on nutrient agar, bacterial isolates were examined by gram staining. Viable count was done to count the live bacteria and ten fold serial dilution was used.

Isolation of fungi was done on saboured agar. The contaminants were drawn from the apple surface with sterilized loop from punctures, cut and infected parts and applied by streaking method. A total of thirty six fruit samples were inoculated on Petri dish with moist cotton to maintain high relative humidity and incubated at 25°C for 3-4 days. The fungal isolates were examined by their morphological characteristics. Eight apples from each variety were used to determine the effect of washing with cold running tap water. The washing time for each variety was 1, 2, 3 and 4 minutes and microbial count was determined by inoculating the samples on nutrient agar at 37°C for 24 hours.

One way Analysis of Variance technique was applied to know the difference due to varieties in chemical composition of apple flesh. Two way Analysis of Variance was used to describe the effect of apple sources on bacterial growth and varieties. Proper tabulation and Mean \pm SE were worked out.

RESULTS AND DISCUSSION

To determine the nutritional potential of apple flesh, it was chemically analyzed. The composition of flesh of different varieties of apple for gross energy, moisture, carbohydrate, protein, fat and ash is shown in Table 1. The gross energy contents of Red delicious, Kala kulu and Golden delicious were 69.54, 56.77 and 73.28 kcal/100g, respectively. It was observed that the mean moisture contents in different varieties were close to each other (83.34-85.97 %). Watt and Merrill (1950) reported that apple of different varieties had moisture value as 84.1 percent. The carbohydrate, protein and fat contents were higher in Golden delicious variety. The overall ash contents ranged from 1.1- 5.0 present in all three varieties. Podsedek *et al.* (2004) determined the

proximate composition of edible portion of apple and in different apple varieties the percentage of water was 84.1, carbohydrate 14.9, protein 0.3, ash 0.29 and fat 0.4. The differences in the above report with the results of the present study could be due to difference in varieties, availability periods and environmental factors.

The volume of the juice extracted and its pH with and without peels are shown in Table 2 and 3. The apples of Red delicious variety were usually large in size and were juicier than other two varieties. The percentage of juice was therefore, higher in this variety. The volume of juice extracted with peels was higher than juice extracted without peels. The hand peeling removes some volume of flesh along with peels and that probably decreased the volume of juice. Marlett (2000) found that peeling reduced apple juice concentration about 15 % by decreasing apple flesh wasted through peeling. The pH of apple juice of all the varieties was acidic. Among three apple varieties, the most acidic variety was Red delicious. The pH values of juice extracted before peeling of all the three varieties ranged from 4.13-5.21 and the pH values of juice extracted after peeling ranged from 3.98-5.0. The pH of juice of all varieties was more acidic when it was extracted without peels. This could be due to certain compounds in the peels that reduced the acidity. Karaibrahimoglu *et al.* (2004) determined pH values of apple juice from Red delicious and Golden delicious and it ranged from 4.1-4.9 with peels.

After weighing of 315 samples, 105 for each variety, these were graded on the basis of their weight. In all three varieties, the highest weights of large, medium and small sized apple belonged to Red delicious variety, Kala kulu ranked second while Golden delicious had the lowest weights. The percentage of flesh, peels and seeds of apples have been presented in a pie diagram in Fig I. It may be seen that Red delicious had almost 98 percent edible portion (flesh + peels). While in Kala kulu and Golden delicious varieties, the edible portions were 96 and 93 percent, respectively.

Microbiological examination included the identification of bacteria and fungi. The bacterial growth characteristics along with viable colony count in different apple varieties are shown in Table 4. Red delicious variety showed variability among samples purchased from different markets and retail outlets. All the three samples from superstore and supermarket level had gram +ive staining characteristics. In city market two out of three samples and in local mundes all samples had gram -ive staining. Viable count ranged from 90 X 10⁵ to 288 X 10⁵/ml. Similar results were obtained in case of Kala kulu variety where viable count ranged from 234 X 10⁵ to 340 X 10⁵/ml. In Golden delicious variety, 50 percent samples had gram +ive bacterial growth. Viable count among varieties was very variable. Bacterial growth was highly dependent on the sources and varieties of the apple. Abadias *et al.* (2006) concluded that gram -ive bacteria

Table 1 Mean (\pm SE) percent proximate components of flesh of different apple varieties

Apple Variety	No of samples	Energy Kcal/100g	Moisture	Carbohydrate	Protein	Fat	Ash
Red delicious	7	69.54 \pm 5.14 ^{ab} (55.33-101.07)	83.34 \pm 0.83 (75.67-85.65)	9.25 \pm 2.05 (2.64-19.35)	2.25 \pm 0.15 (1.64-2.73)	1.95 \pm 0.54 (0.90-5.00)	3.18 \pm 0.55 ^b (1.80-5.0)
Kala kulu	7	56.77 \pm 5.03 ^a (37.6-84.78)	85.97 \pm 1.47 (86.42-87.56)	8.44 \pm 0.74 (5.19-11.80)	2.18 \pm 0.13 (1.64-2.73)	1.00 \pm 0.32 (0.40-2.50)	2.54 \pm 0.38 ^{ab} (1.20-4.80)
Golden delicious	7	73.28 \pm 3.09 ^b (63.43-88.77)	84.58 \pm 0.82 (83.16-86.69)	9.46 \pm 0.82 (6.53-12.18)	2.33 \pm 0.12 (2.18-2.73)	2.21 \pm 0.57 (0.60-4.40)	1.50 \pm 0.10 ^a (1.10-1.70)

The values in paranthesis are ranges. Same alphabets on means in a columns show non- significant differences

Table 2 Mean (\pm SE) weights of an apple, volume of juice and pH of different varieties with peels

Apple variety	Samples	Weight /Apple (grams)	Juice volume mL/apple	Percentage of juice extracted	Juice pH
Red delicious	7	266.83 \pm 14.05 (192.0-310.0)	150.66 \pm 9.89	56.21	4.13
Kala kulu	7	166.71 \pm 22.82 (86.6-261.5)	82.92 \pm 12.70	49.81	5.21
Golden delicious	7	149.12 \pm 16.02 (103.5-215.5)	64.28 \pm 6.89	43.43	5.14

Table 3 Mean (\pm SE) weights of an apple, volume of juice and pH of different varieties without peels

Apple variety	Weight /Apple (grams)	Weight of flesh (grams)	Juice volume mL/apple	Percentage of juice extracted	Juice pH
Red delicious	253.57 \pm 12.71 (210-304.5)	213.14 \pm 10.85 (184.0-261.5)	121.5 \pm 6.72	47.72	3.98
Kala kulu	179.0 \pm 24.73 (103.6-240.5)	154.57 \pm 22.30 (87.40-209.0)	60.92 \pm 10.95	38.19	4.90
Golden delicious	169.0 \pm 20.24 (101.5-253.5)	135.32 \pm 17.15 (82.90-201.50)	53.64 \pm 9.29	39.32	5.00

Table 4 Bacterial growth characteristics and viable count of different apple varieties

Sources	No of sample	Varieties					
		Red delicious		Kala kulu		Golden delicious	
		Bacterial growth	Viable count	Bacterial growth	Viable count	Bacterial growth	viable count
Superstore (cold stored apple)	3	Gram +ive,	280x10 ⁵	Gram +ive,	240x10 ⁵	Gram+ive,	296x10 ⁵
		Streptococci	190 x10 ⁵	Streptococci	240x10 ⁵	Staphylococci	298x10 ⁵
			280x10 ⁵	Staphylococci	296x10 ⁵	Streptococci	310x10 ⁵
Supermarket (sudar mandi)	3	Gram+ive,	190x10 ⁵	Gram+ive,	340x10 ⁵	Gram+ive, bascilli	560x10 ⁵
		Steptococci	190x10 ⁵	Staphylococci	340x10 ⁵	Gram-ive,bascilli	349x10 ⁵
		Streptobascilli	230x10 ⁵		340x10 ⁵	Gram-ive,disperse	390x10 ⁵
Market (city markets)	3	Gram+ive	90x10 ⁵	Gram+ive,	296x10 ⁵	Staphylococci	10x10 ⁰
			120x10 ⁵	Gram-ive, strepto	280x10 ⁵	Gram+ive	79x10 ⁵
			120x10 ⁵	Staphylococci and disperse	296x10 ⁵	Staphylobascilli	123x10 ⁵
Local mundes (retail outlets)	3	Gram-ive, bascilli	120x10 ⁵	Gram-ive,	231x10 ⁵	Staphylococci	180x10 ⁵
		Cocci and	120x10 ⁵	Streptococci and	256x10 ⁵	Gram-ive,	180x10 ⁵
		dispersed	130x10 ⁵	dispersed	235x10 ⁵	Staphylococci	180x10 ⁵
						Bascilli and dispersed	241x10 ⁵

were pre-dominantly present on apples. In the present study out of 36 samples, 21 were gram +ive and 15 were gram -ive. This showed that on apple surface the most dominant strains were gram +ive. This difference in results may be due to difference in pre and post harvest conditions. The wounds, cuts and punctures on apple surface break the external barrier (apple peels) and once this external barrier is broken, it creates an opportunity for bacterial colonization (Janisicwicz *et al.*, 1999).

Fungal contamination of different types of fungi present on the surface of three apple varieties has been presented in Table 5. The most common genus of fungi grown on the surface were *Aspergillus*, *Mucor*, *Rhizopus* and *Pencillium*. While some samples did not show any growth of fungi. Chen *et al* (2004) stated that among

microbes *Aspergillus* and *Penicillium* were the most dangerous species of fungi, producing toxin namely aflatoxin and patulin. Jackson *et al* (2003) reported that patulin is a mycotoxin produced primarily by *Penicillium expansum*. Randomly selected samples from each variety were subjected to washing treatment at different time interval. Samples were then washed with sterilized normal saline and bacterial count was determined by inoculating the samples on nutrient agar. Washing apple under running tap water at different time interval resulted in significant reduction of bacterial attachment (Fig II). Buchanan *et al* (1999) observed that apples washed subsequently by 1- min tap water rinse, reduced pathogen levels by 1- to 3-log cycles but did not eliminate the microorganism, particularly from outer core region.

Table 5 Number of samples found contaminated with different genus of fungi on apple varieties.

Apple Variety No. of samples	No. of samples contaminated with fungi				
	Aspergillus	Mucor	Rhizopus	Penicillium	No Growth
Red Delicious 12	6	4	3	2	4
Kala Kulu 12	4	5	2	1	5
Golden Delicious 12	1	6	5	2	4

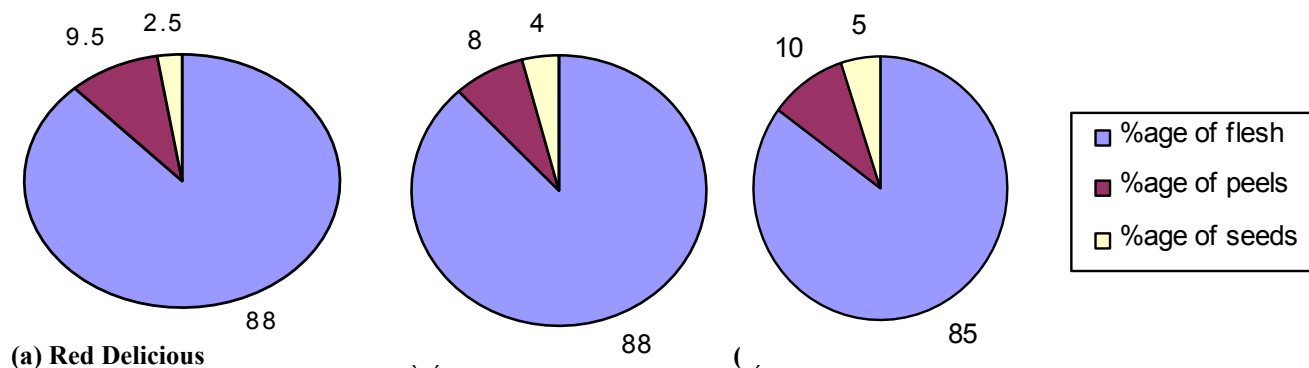


Fig. 1 Percentage of flesh, peels and seeds of different apple varieties.

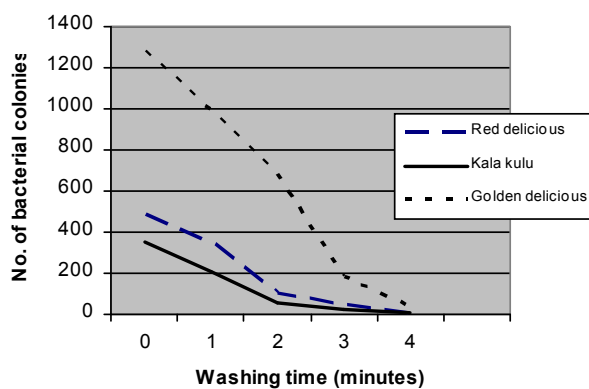


Fig II. Graph line showing decreasing trend of bacterial count by washing for different ti

Conclusions: The results of apple grading revealed that among three varieties, Red delicious contained most of the large sized apples, Golden delicious ranked in the category of small sized apples while Kala kulu contain medium sized, peeling reduced both volume and pH of apple juice extracted, Apple flesh had higher energy contents of Golden Delicious variety. Kala Kulu variety had higher moisture contents. While carbohydrate, protein and fat contents were high in Golden Delicious variety, significant amount of bacteria and fungi has been associated with apple varieties, careful culling of apples before purchasing is recommended because damaged, cuts and punctures on surface create an opportunity for bacterial colonization and Washing with water alone for 3 to 4 minutes under running tap water reduced the recoverable population of microbes from starting population. The apples when consumed raw should

invariably be washed with running cold water for 3 to 4 minutes.

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