

# Differentiation of Some Fresh Meat Species and Their Corresponding Frozen Minced Products Using Visible / Near-Infrared Reflectance Spectroscopy

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## ABSTRACT

The authenticity and detection of adulteration of meat and meat-related products are major issues in the food industry. A visible-near infrared spectroscopic method as a rapid and easy tool was used to differentiate between seven fresh meat samples and their corresponding frozen minced products of different species (ostrich, beef, buffalo, goat, mutton, camel and pork). A derivative treatment of reflectance spectra improved the classification of all meat species. All the investigated meat samples showed a nearly similar spectral pattern and absorption bands except for ostrich meat. Freezing treatment caused a pronounced increase in the percentage reflection (R%) of mutton and buffalo meat, a decrease in pork and a slight response in ostrich, goat and camel meat.

**Keywords:** adulteration, color parameters, identification, meat speciation, visible-near infrared spectroscopy

## INTRODUCTION

The present work constitutes an attempt to differentiate between several freshly minced meat species and to probe the effect of freezing treatment using visible/near-infrared (NIR) reflectance spectroscopy as a rapid tool for detecting the main functional groups as well as measuring color parameters of the tested minced meat samples. By doing so, more light can be shed on the identification and adulteration of these meat species.

Meat is a very heterogeneous product since the chemical composition, technological and sensory attributes are highly influenced by pre-slaughter (breed, gender, age, mass and environment) and post-mortem factors (storage time, temperature) (Andrés *et al.* 2007; Prito 2009). Therefore, the variability in quality characteristics of meat is the consumer's main concern (Leroy *et al.* 2003; Warriss 2004).

Meat from different species is not easy to distinguish visually, especially when they are deboned and frozen in large blocks. Hence inter-species adulteration i.e. substitution of expensive meat with cheaper alternatives, may occur (Barai *et al.* 1992). Partial substitution of high-value meat with one of lower value or quality is considered an adulteration and may pose religious or potential health problems. While speciation is apparent when meat is examined in large pieces, after mincing, it becomes difficult to establish species without a sophisticated analytical procedure (McElhinney *et al.* 1999a).

The determination of food authenticity and the detection of adulteration are major issues in the food industry. Authentication methods can be categorized into areas where fraud is most likely to occur: origin, substitution, processing treatment and addition of non-meat ingredients. Analytical methods used in authentication are as diverse as the authentication problems, and include a diverse range of equipment and techniques (Ballin 2010).

With meat and meat products major authenticity issues concern the substitution of high-value raw materials with cheaper materials such as less costly cuts, mechanically

recovered meat, offal, blood, water, eggs, gluten or other proteins of animal or vegetable origin (Hargin 1996; Al Jowder *et al.* 1997; Cordella *et al.* 2002; Al Jowder *et al.* 2002). For example, ostrich meat, which is similar in taste and texture to veal and beef, is presented as a new red meat alternative and a health food (Thawatchai and Arunee 2007). In Egypt, although ostrich meat is expensive, demand is high. Hence, substitution of expensive meat with cheaper alternatives may occur. So, there is need for a rapid and easy method for meat species identification in order to detect such fraud.

Meat speciation has been addressed by immunological and enzymatic procedures as well as electrophoretic techniques (DNA and PCR-based techniques) used to differentiate fresh from frozen meat (Patterson and Jones 1990; Smith 1991; Sharma *et al.* 1994; Sieberte *et al.* 1994). These methods are cheap and have the ability to detect a wide range and low levels of adulteration. However, spectroscopic methods are attractive options due to their speed of analysis and minimal sample preparation (Cozzolino *et al.* 2004).

Near infrared reflectance (NIR) spectroscopy is one of the most efficient and advanced tools for the estimation of quality attributes in meat and meat products (Osborne *et al.* 1993; Liu *et al.* 2003; Prito *et al.* 2009). Also, NIRS technology has been used the meat industry to determine quality traits such as chemical composition, water holding capacity, heme pigment content and color, Warner-Bratzler shear force and sensory tenderness in beef and applied to determine the total fat and fatty acid composition in foods, without previous fat extraction or treatment of samples (Sierra *et al.* 2008). Moreover, NIR has been successfully applied to the quantitative determination of major constituents (moisture, fat and protein) in meat and meat products and is approved by the AOAC (Anderson 2007) using FOSS artificial neural network (ANN) prediction models.

The discrimination between frozen and unfrozen beef (Downey and Beauchene 1997; Thyholt and Isaksson 1997), beef and kangaroo meat (Ding and Xu 1999; McDevitt *et al.*

2005) as well as ostrich meat (Viljoen *et al.* 2005) has been possible using NIR and provides complete information about the molecular bonds and chemical constituents in a sample scanned, so it is a convenient tool not only for characterizing meat-based foods, but also for quality measurements and process control.

McElhinney *et al.* (1999b) carried out the quantization of lamb content in mixtures with raw minced beef using visible near and mid-infrared (MIR) spectroscopy. Chemometric processing of visible and NIR spectra for species identification in selected raw homogenized beef and lamb meats has been investigated by McElhinney *et al.* (1999a).

$L^*$ ,  $a^*$  and  $b^*$  color of both intact and homogenized pork muscle (Cozzolino *et al.* 2003), beef longissimus thoracis (Leroy *et al.* 2003), beef steaks (Lui *et al.* 2003), beef (Andrés *et al.* 2008) and adult steers and young cattle meat (Prieto *et al.* 2008) could successfully be predicted using NIR spectroscopy. However, Prieto *et al.* (2009) reported that NIR had a limited ability for estimating technological and sensory attributes, including color parameters, which may be mainly due to the heterogeneity of meat samples and their preparation.

## MATERIALS AND METHODS

### Meat samples

Fresh ostrich (*Struthio camelus*), beef (cow: *Bos taurus*), buffalo (*Bubalus bubalis*), mutton (sheep: *Ovis aries*), goat (*Capra hircus*), camel (*Camelus dromedarius*), and pork (pig: *Sus Scrofa domestica*) meat was purchased from a local market in Cairo, Egypt. Skin, bone, fatty tissue, connective tissue and visible bleeding tissue were removed from each sample to ensure the highest possible quality of lean meat. Each sample was minced twice using a meat grinder (Sanyo Meat Grinder MG 2000, Sanyo Electric Co. Ltd., Japan). The homogeneously minced meat samples of each species were divided into two portions. Each sample of the tested minced meat was packed into a polyethylene bag. The first portion was refrigerated for 12 h at 4°C before NIR and color measurements. The second portion was frozen and stored at -18°C for 21 days before spectroscopic analysis.

### Visible/NIR measurements

Visible/NIR reflectance spectra of the minced meat samples under investigation were measured using a Jasco Model V-570 UV/VIS/NIR spectrophotometer at room temperature. Reflectance measurements were carried out using an instrument equipped with an integrated sphere, enabling direct measurement of specular reflection. The instrument had a 0.1-nm resolution and wavelength accuracy of +0.3 nm (at a spectral wavelength of 0.5 nm). Each sample was measured by the spectrophotometer in triplicate.

### Color measurements

Color analysis was carried out using a Hunter Lab. scan XE colorimeter (Hunter Lab. Inc., Reston, VA, USA) with  $\lambda = 400-700$  nm. The instrument was standardized prior to each use with a white tile, with the following values:  $X = 77.26$ ,  $Y = 81.94$  and  $Z = 88.14$ . Commission International d'Eclairage (CIE):  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) saturation index were measured. Reflectance measurements were collected at 10-nm increments using illuminate A (Podolak *et al.* 1997). Three random readings per sample were obtained.

### Statistical analysis

Standard error (SE) and least significant difference (LSD) were determined using the methods described by Snedecor and Cochran (1980). Classification accuracy was calculated against the original identity of the samples; second derivative operation was developed with computer software (SAS 1990).

## RESULTS AND DISCUSSION

### Visible/NIR spectra

Average reflectance spectra of seven fresh meat samples and their corresponding frozen minced samples of different animals (ostrich, beef, buffalo, goat, mutton, camel and pork) are shown in **Fig. 1**. All the investigated samples showed a nearly similar spectral pattern and absorption bands expect the ostrich meat sample. In the NIR visible region (~ 400-850 nm), six meat samples (beef, buffalo, goat, mutton, camel and pork) showed nearly the same spectral pattern and absorption bands; however, the most intense out-standing absorption band in this region was around the ~790-870 nm region for these six samples. In contrast, in the 400-2500 nm region the differences in the spectral pattern and the shift of absorption bands were more pronounced for the two ostrich samples (i.e., fresh or frozen). Moreover, all the investigated fresh minced meat samples showed numerous absorption peaks (fine to medium structures) especially around the 1800-2500 nm region accompanied by remarkable differences in energy reflectance. Ding and Xu (1999) reported that the difference in energy reflectance between minced beef and kangaroo meat may be due to the light-scattering effect resulting from variations in particle size. In most cases of NIR spectroscopic analysis, scattering effects need to be corrected. NIR spectra of foods comprise broad bands arising from overlapping absorptions corresponding mainly to overtones and combination of vibrational modes involving C-H, O-H and N-H chemical bonds (Osborne *et al.* 1993).

### Functional groups

**Fig. 2** shows bands around the 2230-2210 nm region, probably due to C-H groups, suggesting that differences in polyunsaturated fatty acids may contribute to different NIR patterns in minced meat of different origin. Varnam and Sutherland (1995) showed implicit differences in the chemical composition of beef and lamb; these differences could be detected by spectroscopic techniques (NIR). It is generally accepted that lean meat composition of beef and lamb are relatively constant with the major source of variation related to lipid content.

The characteristic functional groups of protein, fat and water content in the tested fresh and frozen minced meat samples were clearly detected by NIR, as shown in **Fig. 2**. Thus, the bending and stretching absorption bands for protein (-NH and -NH<sub>2</sub> groups) were revealed at 810, 1100, 1544, 1922, 1974 and 2140 nm; for fat (-CH groups: 1<sup>st</sup> and 2<sup>nd</sup> overtones) at 907, 1025 and 2360 nm and for water (-OH group) at 938 (-OH 2<sup>nd</sup> overtone), 1422 (-OH 1<sup>st</sup> overtone) and 1974 nm (-OH combination tone). Also, by comparing the fresh and frozen minced meat samples, it was found that the reflected energy of frozen meat was less than the fresh meat samples, except for pork. Presumably, this difference in energy reflectance may be due to a light scattering effect resulting from variations in particle size. McElhinney *et al.* (1999a) pointed out that the variation in particle size of samples were likely due to differences in texture. Thus, this apparent differentiation may be a matter of physical effects (particle size distribution, sample packing) rather than differences in chemical species. In the present study, major differences between the obtained spectra involved a base line shift induced by the frozen state were clearly evident in the second derivative plot of the same spectra.

### Interaction effects of different meat species on reflection (R) percentage

Statistical analysis of the data for interaction effects of different species of meat and freezing treatment on reflection (R) percentage i.e., R%, are recorded in **Table 1**. The highest mean R% of the fresh minced meat was for pork and the

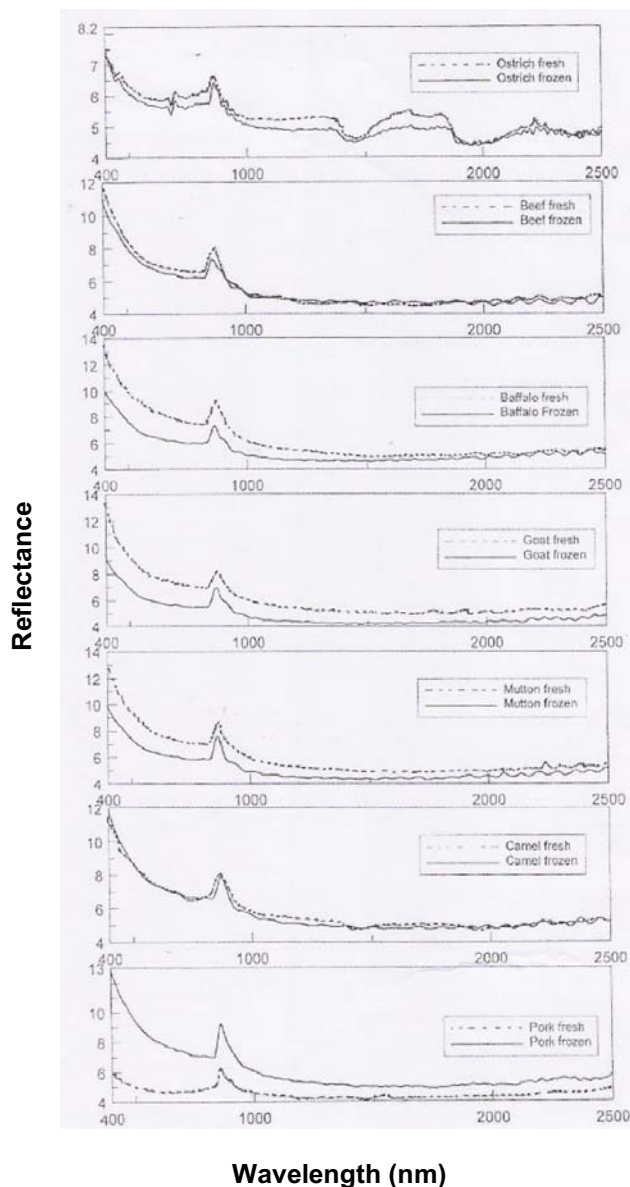


Fig. 1 Average reflectance spectra of representative meat samples.

lowest was for ostrich meat. The differences in R% within the investigated meat species were significant except between mutton and goat, which was non-significant. On the other hand, R% of different fresh meat species could be arranged in descending order: pork (9.197) > camel (8.041) > goat (7.554) > buffalo (7.177) > beef (7.005) > mutton (6.617) > ostrich (6.277). A wide difference (46.519%) was detected between R% of pork and ostrich meat.

Regarding the mean R% of frozen meat species, the highest percentage was obtained by buffalo meat followed by camel meat while the lowest R% was shown by pork followed by ostrich meat. Furthermore, the differences between ostrich meat and pork exceeded those between beef and mutton. The R% of the above-mentioned frozen meat species could be arranged in descending order: buffalo (8.934) > beef (8.700) > camel (8.658) > goat (8.187) > mutton (8.014) > ostrich (6.712) > pork (6.539).

Concerning the interaction effect of freezing treatment on the R% of different meat species, generally, the data showed that the R% of frozen meat was more than that of fresh meat samples, except for pork, where the reverse was true. This treatment raised the R% of ostrich, beef, buffalo, mutton, goat and camel by 6.93, 24.20, 24.48, 21.11, 8.38 and 7.67%, respectively. Reversibly, freezing treatment lowered R% sharply, by 40.65% compared to the fresh sample in the case of pork. This means that freezing induced a pronounced increment (positive effect) on beef, buffalo and

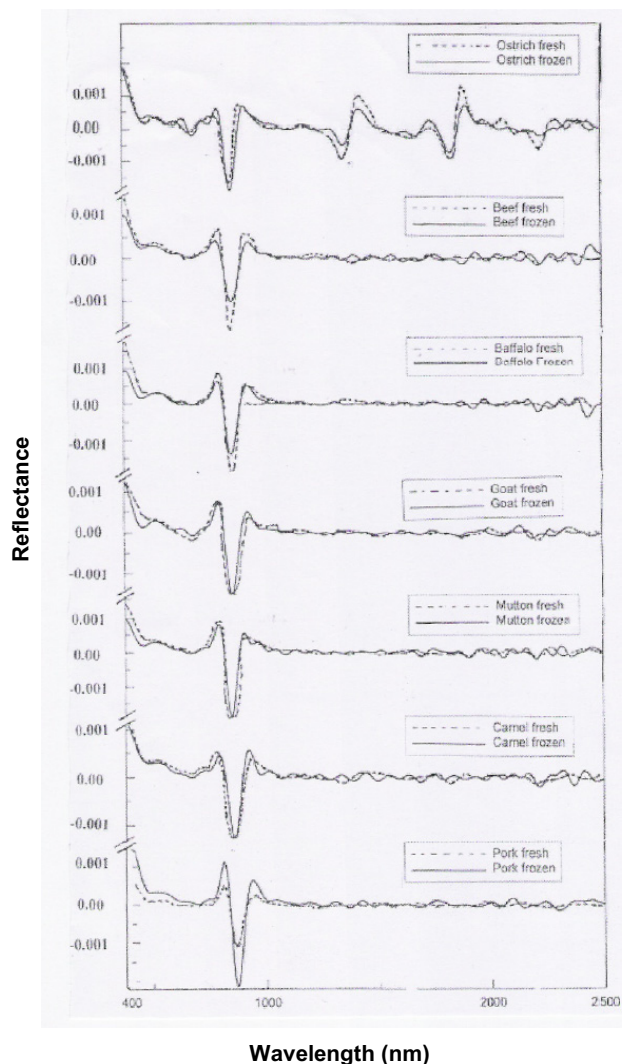


Fig. 2 Second derivatives of representative meat samples.

mutton but it caused a decrease (negative effect) in pork. Meanwhile, the R% increment of ostrich, camel and goat meat responded slightly to freezing, ranging between 6.93 and 8.38%.

From the aforementioned data it can be concluded that fresh minced ostrich meat and pork R% were around 6% in the 856-866 nm region and around 9% in the 862-864 nm region; however, after freezing both meat samples showed an R% around 6% in the 856-864 nm region. R% of the fresh beef, buffalo, mutton and goat meat samples were around 7% in the 860-888 nm region while fresh camel meat was around 8% in the 862-872 nm region. After freezing all R% values of the investigated samples were around 8% in the 860-874 nm region. These findings support the possibility of using visible and NIR measurements as an effective and simple test to identify and differentiate between different meat species.

### Color evaluation

Color is an important contributing factor for the classification of meat since the spectral classification of meats may be due to physical and chemical differences.

The NIR forms that part of the electromagnetic spectrum in the wavelength range of 780 to 2500 nm. Thus, wavelengths below 700 nm are not within the NIR spectral range and it is apparent that color changes influence the discrimination process. Color information alone (650-748 nm) is, however, insufficient to achieve useful levels of discrimination (Osborn *et al.* 1993).

As shown in Fig. 2 there are four bands (as fine to medium structures) at approximately 430, 545, 575, and

**Table 1** Reflection of visible and NIR spectra of fresh and frozen minced meat samples of different species.

Treatment	Meat samples															
	Ostrich		Beef		Buffalo		Mutton		Goat		Camel		Pork		Mean values	
	R%	VT	R%	VT	R%	VT	R%	VT	R%	VT	R%	VT	R%	VT	R%	VT
Fresh meat Samples	6.277	14.50	7.005	15.38	7.177	15.57	6.617	15.62	7.554	15.96	8.041	16.50	9.197	17.66	7.553	15.88
Frozen meat Samples	6.712	15.04	8.700	17.15	8.934	17.39	8.014	16.42	8.187	16.64	8.658	17.23	6.539	14.81	7.963	16.38
Mean values of meat Species	6.500	14.77	7.853	16.27	8.056	16.48	7.816	16.02	7.871	16.30	8.350	16.870	7.868	16.24		

R% = Reflection value  
V.T. = Values of percentages transformed into degrees of an angle (Steel and Torrie 1981)  
L.S.D. + Fisher values for:  
Species : 0.354  
Freezing: N.S  
Interaction: 0.783

**Table 2** Color parameters values of the tested fresh and frozen meat samples of different species.

Color parameters	Meat samples							
	Ostrich	Beef	Buffalo	Mutton	Goat	Camel	Pork	
<b>Fresh samples</b>								
L* (lightness)	32.29 ± 0.21	37.41 ± 0.52	33.05 ± 0.03	45.45 ± 0.81	40.56 ± 1.91	32.60 ± 1.85	33.56 ± 0.5	
a* (redness)	16.74 ± 0.32	19.79 ± 0.96	12.90 ± 0.48	11.09 ± 0.67	10.91 ± 0.68	15.08 ± 0.68	14.41 ± 0.20	
b* (yellowness)	14.27 ± 0.23	17.54 ± 0.85	12.29 ± 0.40	12.16 ± 0.97	9.83 ± 2.66	11.22 ± 0.50	11.72 ± 0.22	
<b>Frozen samples</b>								
L* (lightness)	31.78 ± 0.01	37.16 ± 0.50	32.96 ± 0.68	44.98 ± 0.73	36.90 ± 3.39	30.77 ± 0.64	31.34 ± 1.48	
a* (redness)	16.06 ± 0.002	14.13 ± 0.24	10.88 ± 0.46	11.59 ± 1.22	12.86 ± 0.99	14.06 ± 2.31	15.06 ± 0.28	
b* (yellowness)	15.63 ± 0.00	12.28 ± 0.19	12.41 ± 1.90	14.03 ± 1.54	10.70 ± 0.95	11.51 ± 1.58	14.07 ± 0.51	

635 nm in the visible region (400-750 nm), and two other broad bands around 760 and 980 nm in the NIR region (750-1100 nm). These spectral features in the visible region are nearly similar to those of chicken meat reported by Liu *et al.* (2003); this is due to the fact that both beef and chicken contain myoglobin protein, which is the primary heme pigment responsible for the color of meat.

Leroy *et al.* (2003) reported that the NIR spectra collected on fresh beef (*longissimus thoracis*) showed good potential to predict CIE L\* and b\* parameters in reflectance mode. The mincing of meat samples before taking spectra could help to reduce heterogeneity. Moreover, Prieto *et al.* (2008) showed correlation coefficients up to 0.8 between absorbance data and L\* and b\* color parameters at 1230-1400 and 1600-1710 nm, which correspond to C-H second overtone and C-H combination bands, and C-H first overtone, respectively. The same authors indicated that these wavelengths are related to the absorbance of the C-H bonds present in the intramuscular fat.

Previously, the visible and NIR region enabled a\* prediction for pork and beef meat in the studies carried out by Cozzolino *et al.* (2003) and Liu *et al.* (2003). It is well known that a\* is related not only to the water content of meat but also to the concentration of myoglobin and the relative proportions of its three derivatives; therefore, the rates of meat discoloration can be assayed by measuring reflectance differences in the visible region of the spectra. Also, failure of NIR spectroscopy to estimate L\*, a\* and/or b\* color values in beef, pork and poultry meat has been previously noted (Prieto *et al.* 2009). Moreover, the same authors reported that NIR showed limited ability for estimating technological attributes which may be mainly due to the heterogeneity of the meat samples and their preparation.

Instrumental Commission International de l'Eclairage (CIE) L\* a\* b\* color measurements can provide reliable information about meat quality (Lyon and Lyon 1991). In the present study, these three color parameters for fresh and frozen minced meat samples of different species were determined and compared. The data in **Table 2** showed obvious differences in color parameter values for the investigated fresh minced meat samples of different species.

Fresh mutton meat sample had the highest L\* value (45.45 ± 0.81) followed by goat, beef and then pork values (40.56 ± 1.91, 37.41 ± 0.52 and 33.56 ± 0.15, respectively) while ostrich and camel meat samples attained the lowest

L\* values.

Regarding the redness (a\*) color parameter, the data in **Table 2** indicates that fresh buffalo, mutton and goat meat samples have the lowest a\* values (ranging from 12.90 ± 0.48 to 10.91 ± 0.68). Fresh beef and ostrich meat samples had higher a\* values than the corresponding values of camel meat and pork samples.

Fresh beef had the highest yellowness (b\*) value followed by ostrich among the all studied meat samples. However, the lowest b\* value was for goat meat.

In general, fresh mutton minced meat had highest lightness (L\*) and goat sample had lowest redness (a\*) values. On the other hand, fresh beef meat sample had a higher a\* value than all other fresh meat samples. Beef was characterized by high a\*, b\* and L\* values compared to ostrich meat. Moore (1990) previously reported that many factors influence the color of meat and meat products including pigment concentration, exposure to oxygen, the amount of moisture retained in muscle fibers, muscle fiber type, muscle pH and age of the animal. Also, Renner (1990) concluded that the surface color of meat depends on the quantity of myoglobin present, its chemical state and also on the chemical and physical conditions of other components in meat. Meat myoglobin content varies with species and also with age, sex and physical activity as emphasized by Hedrick *et al.* (1994) and Lawrie (1998).

The interaction effect data of freezing treatment (21 days at -18°C) on the different meat species (**Table 2**) points out a slight decrease in the L\* value most of the tested samples except for pork and goat meat, which showed a marked decrease in percentage (6.62 and 9.02%, respectively). Meanwhile, a\* values exhibited a remarkable increase in frozen goat meat (17.87%). This parameter in beef and buffalo meat decreased markedly by freezing and this decrease in beef exceeded that in buffalo meat by 12.94%.

The a\* value of ostrich meat and mutton (either fresh or frozen) samples seemed almost identical (**Table 2**). This indicates that freezing caused a slight effect in the a\* values for these two meat types.

The percentage increase in b\* can be depicted in ascending order: goat (8.85%) < mutton (15.38%) < pork (20.05%); however, for beef and ostrich meat, the opposite was found. The values of b\* for both fresh and frozen camel and buffalo meat seemed to be slightly affected.

The above-mentioned findings for differences in color

parameters of the studied minced meat samples (fresh and frozen) may be attributed to many factors. Juncher *et al.* (2001) reported that factors affecting surface color are related to differences between breeds and even individual animals, the age, chilling process, method of packaging, retail light exposure, and time or temperature regime during storage.

## CONCLUSIONS

Based on the above mentioned data of visible/NIR spectra and color parameters of the investigated minced meat species (either fresh or frozen); it can be concluded that visible/NIR spectroscopy and color measurements can be used as rapid, easy and promising tools to differentiate between various minced meat species, identify main functional groups and color parameters, and hence can generally be used to identify meat species and avoid their adulteration.

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