CHANGES IN FUNCTIONAL PROPERTIES AND SDS – PAGE PATTERN OF GOAT MEAT PROTEINS DURING FROZEN STORAGE

S. Shaghaghian,[1], Abedi, A.[1], M. Aminlari, [1,2]*, and R. Ramezani[1]

[1] Department of Food Science and Technology, School of Agriculture and [2] Department of Biochemistry, School of Veterinary Medicine, Shiraz University, Shiraz, 71345 IRAN

* Corresponding author: E-mail: aminlari@shirazu.ac.ir, Fax: 00987002286940

Abstract: ***Goat meat has an enormous potential in meat product fabrication. An understanding of the functional properties of goat meat proteins is necessary to utilize it as fresh or in processed products. The aim of this study was to investigate the changes in protein solubility, water holding capacity, and protein electrophoretic pattern of goat meat during freezing. Small pieces of fillet muscles of goats were packed in polyethylene bags and kept at – 18 °C. Nitrogen solubility index, NSI), and water holding capacity (WHC) were determined at one week intervals. Also, extracted proteins were subjected to SDS–PAGE. Results showed a significant decrease in NSI and WHC during frozen storage such that after 12 weeks NSI and WHC dropped to 15% and 30% of their original values, respectively. No significant change in SDS–PAGE patterns was observed.***

Index Terms**: Goat meat, freezing, SDS–PAGE, protein solubility, water holding capacity.**

I. INTRODUCTION:

Meat as a rich source of proteins, vitamins, minerals and essential amino acids plays an important role in man's diet (Azad & Akter, 2005; Strasburg, et al, 2008). The average annual red meat production in Iran is 8000 tons and per head consumption is 11.5kg per year. In Iran people mostly prefer to eat mutton (Hashemi, 1995). Goats are an important nutrient source mostly in rural areas. According to Iran statistics, in the year 2003, the number of goats and kids reached 26 million which occupies the second rank after chicken (Hashemi, 1995). Goat is an important source of meat For people in the technologically developing regions, situated mainly in the tropics. These regions account for more than 90% of the estimated world goat population of 504 million, with approximately 56% in Asia, 33% in Africa and 7% in South and Central America and the Caribbean (FAOSTAT, 2005; Norman, 1991). The importance of goats as meat–producing animals varies world wide. The dependence of humans on goats was illustrated by French (1970) and Norman (1991). They calculated ratios of humans to goats for various world regions from FAO statistics. The ratio was 3:1 for Africa as a whole and 4.05 for southern Africa, 10:1 for Asia and 12:1 for Latin America. On a global scale goats provide the least meat per caput, being 0.5 kg per caput, compared with beef at 10.1 kg, pork at 12.7 kg, sheep at 1.3 kg and poultry at 7.2 kg. In terms of world regions the provision by goats is the highest in Africa, 1.04 kg per caput, followed by Asia, 0.47 kg per caput (French, 1970; Norman, 1991; Dhanda, et al, 2003).

In many countries, goats are primarily used for textile and milk industries, and meat is considered as a secondary product (Totosaus, et al, 1998). However, goats present various advantages as meat producing animals compared to other species including: their high reproductive rate, short interval between pregnancies, resistance to harsh climates and small carcasses, easy to preserve and fast to be consumed (Totosaus, et al, 1998). The use of this sub-employed protein source in meat product fabrication has an enormous potential (Smith, 1988). Goat meat is preferred to sheep, because it has lower fat and cholesterol content like poultry (Gaili, & All, 1985). On the other hand, it is richer than poultry in mineral and poorer than other meat in saturated fats. Goat meat is therefore classified as a white meat. In contrast with goat meat, goat kid meat has especial taste and aroma. It tastes delicious like lamb (Schonfeldt, 1989). Several studies have indicated that goat meat is inherently less tender than sheep probably due to a higher collagen content with a lower collagen solubility than lambs (Heinze, et al, 1986).

An understanding of the functional properties of goat meat proteins is necessary to utilize these cheap carcasses, introducing new products, using nontraditional protein sources, and improving existing products (Smith, 1988).

The purpose of this study was to investigate the changes in solubility, water holding capacity color, and protein electrophoretic pattern of goat meat during frozen storage. This information can be useful in developing strategies for storing goat meat and formulation of products made from meat of this species.

II. MATERIALS AND METHODS:

Goat meat was obtained from a local slaughter house. Fillet muscles (*Psoase minor*) were separated, fats and connective tissues removed and divided into 5 g pieces. Each individual sample was packed in polyethylene bags, sealed and kept at – 18 °C. Samples were removed at one week intervals, up to 11 weeks, defrosted at ambient temperature and used for subsequent analyses.

Nitrogen solubility index (NSI) NSI was determined as described by Inkaar & Fortin (1969) and Aminlari, et al, 2009) with some modification. The fresh and frozen meat samples were subjected to SDS-PAGE to determine the effect of freezing on the electrophoretic pattern of the proteins. The supernatants which were used for solubility measurement were also used for SDS–PAGE studies. Slabs for SDS–PAGE were formed according to the discontinuous buffer system of Laemmli (1970). The modified method of Hung & Zayas (1992) was used for determination of Water holding capacity measurement (WHC). Color of frozen meat was evaluated using the method described by Yam & Papadakis (2004).

III. RESULTS AND DISCUUSION

The NSI of fresh goat meat was about 75% (Table 1). A drastic and significant decrease in protein solubility was observed upon storing goat meat at -18°C for different length of time. These changes are more appropriately depicted in Fig.1. A slow and gradual decrease in solubility is observed up to 4 weeks of storage, followed by a rapid decrease thereafter such that after 11 weeks the NSI dropped to 12 %. These changes in NSI is also reflected in changes occurred in WHC. Water holding capacity of fresh meat was o.46 and after 11 weeks storage at -18 °C it dropped to 0.16 (Table 1 and Fig. 2). The supernatants that were used for determination of NSI were also used for SDS–PAGE studies. The results are shown in Figure 3. In general the SDS-PAGE patterns of fresh and frozen meat do not appear to be significantly different. Table 2 shows the changes in color parameters of goat meat stored at -18 °C for 3 weeks. A significant increase in L parameter and decrease in a and b parameters was observed. The color changes after 3 weeks did not significantly change (data not shown). Meat is one of the most concentrated and easily assimilable nitrogenous food that contains those amino acids, polyunsaturated fatty acids as well as vitamins and minerals that are essential for human health and development. Goat meat is an important nutrient source, particularly for people in developing regions of the world where availability of other sources of meat is limited (Dhanda, Taylor, Murray, Pegg & Shand, 2003). General characteristics of goat favoring meat production are: (1) females are early maturing, highly prolific, have good fecundity and mothering ability; (2) goat has an extended breeding season, (3) foraging preferences of goats cause them to graze a wider spectrum of plants than other small stock which accounts for their ability to thrive in adverse conditions; (4) foraging may account for relatively low parasite infestation; (5) goats exploit available feed resources selectively, consuming material with sufficient digestible organic matter at or exceeding their maintenance needs; selectivity and small size enable them to utilize tropical shrub and scrub pastures more efficiently than cattle; (6) goats are generally well adapted to hot environments, tolerating the extremes of desert conditions and high temperature-humidity conditions of the tropics, because of small size, large surface area to body weight ratio, an ability to conserve water, limited subcutaneous fat cover and the particular nature of their coats (Casey, 1992). Unfortunately, lack of an organized goat meat industry and marketing structure in developing countries is primarily responsible for their poor export earnings compared to those in developed countries such as Australia and New Zealand. Goat meat is leaner than meat from other domestic red meat species as well as being comparable in terms of its nutritional constituents (Gaili, & All, 1985; Dhanda). Furthermore, there are virtually no religious or cultural taboos on the eating of goat meat, with the result that goat is readily available to societies in which eating beef, pork or other meat types is prohibited (Gaili, & All, 1985). With an increase in demand by consumers for low-fat red meat alternatives, the future of the goat meat industry looks promising. However, an understanding of the functional properties of goat meat proteins is necessary to utilize this nontraditional protein source (Smith, 1988). Studies on functional properties of proteins and the factors contributing to these characteristics in food systems are essential to determine their potential for use in food products. Protein solubility is a key functional property since proteins generally have to be in solution to exert desirable functional characteristics such as emulsification and gel formation (Feeney, 1977; Lan, et al, 1993; Hwang, et al,

2003; Xiong, 2004; Damodaran, 2008; Aminlari, et al, 2009). Meat product fabrication is based on protein solubilization in salt solution (Hwang, et al, 2003). High solubility is desirable since it allows fast protein dispersion in large quantities, resulting in an efficient diffusion towards water/oil or oil/water interfaces. Environmental conditions, such as pH, temperature, ionic strength and interactions with other meat constituents, significantly influence solubility (Cheftel, et al, 1985; Lan, et al, 1993; Strasburg, et al, 2008). Striated muscle contains 18-20% protein. Meat proteins are divided in three groups by their solubility; sarcoplasmic proteins (soluble in low ionic strength solutions), myofibrillar protein (soluble in high ionic strength solutions) and stroma (insoluble in water or salt solutions) (Hudson, 1992; Xiong ,2004). In the present study, it was shown that the solubility of fresh goat meat protein was about 75 %, a value close to those reported for beef , pork, fish, poultry and buffalo (Khan, Van den berg, & Lentz, 1963; Borchert, Briskey, 1965; King, 1966; Sebranek et al, 1979; Shenouda, 1980; Ziauddin, 1993; Hultin,et al, 1995; Sakata, et al, 1995; Farouk, et al, 2003; Azad & Akter, 2005; Mortensen, et al, 2006; Stika, et al, 2008; Yu, et al, 2009). Most of the decrease in protein content can be attributed to the drip loss after thawing of the frozen meat (Rahelic, & Gawwad, 1985; Ngapo, et al, 1999). However, the solubility drastically decreased during storage with concomitant decrease in WHC and changes in color of the meat. A slow decrease followed by a rapid decline in solubility probably is related to two different phenomena: initial rapid denaturation and aggregation of protein followed by gradual precipitation at later stages. Compared to the effect of freezing on protein solubility of the meat of other species, the data obtained in this study indicates that goat meat becomes remarkably much less soluble than the meat of other species due to freezing (Khan, et al, 1963; Borchert, et al, 1966; Sebranek , et al, 1979; Shenouda, 1980; Ziauddin, 1993; Hultin, et al, 1995; Farouk, et al, 2003; Azad & Akter, 2005; Mortensen, et al, 2006; Yu, et al, 2009). The reason for this difference might be attributed to the difference in the microstructure, higher collagen content and lower collagen solubility (Heinze, et al, 1986). Generally, it has been accepted that the degree of denaturation of meat as a result of freezing is relatively low. Frozen rather than fresh meat is usually used in the meat industry in the preparation of emulsion-style products. Frozen meat is commonly used industrially as raw material for a variety of meat products. Freezing and frozen storage however can produce profound effects on the structural and chemical properties of muscle foods, including changes in muscle fibers, lipids, and proteins, all of which have the potential for significantly influencing the quality attributes of meat and meat products (Matsumoto, 1980; Miller, et al, 1980; Rahelic & Gawwad, 1985; Sakata, et al, 1995; Cofrades, et al, 1996; Ngapo, et al, 1999; Kang, et al, 2008). It is well known that freezing can affect meat through several mechanism: 1- Acceleration and formation of ice crystals which results in rupture of the cells and release of cell constituents (Strasburg, Xiong, & Chiang, 2008); 2- Interaction between proteins and lipid degradation products and denaturation of proteins (free fatty acids, the products of hydrolytic rancidity (Bull and Breese, 1967a,b) and malonaldehyde, a product of polyunsaturated fatty acid oxidation (Buttkus, 1969); 3-Oxidation of sulfhydryl groups to disulfide bonds (Khan, et al, 1963; Hofmann, & Hamm, 1978; Shenouda, 1980; Jiang, et al, 1989) 4- Increase in the rate of chemical reactions due to the concentration effects of freezing, that is, macromolecoules are forced together, (especially actin and myosin) making interactions more probable with resultant denaturation, insolibilization, aggregation, and precipitation of proteins, 5- Reduction in functionality, which negatively affects the quality (texture, water and fat binding properties, sensory characteristics and others) of the final processed products (Hanson and Fletcher, 1985; Carballo, et al, 2000; Moeseke, et al, 2001). It is interesting to note that in this study no significant change in SDS –PAGE pattern of goat meat due to prolonged frozen storage occurred. This observation can be explained as follows: As evidenced in Figure 3, the band related to the myosin heavy chain (MHC~200kDa) is absent in both fresh (lane 2) and frozen samples (lanes 3-8). As explained in the Materials and Method section, electrophoresis was performed on the supernatants of the homogenized samples, prepared for NSI determination. At this stage, it is possible that these proteins precipitated along with stroma proteins and remained in the precipitate after centrifugation. However, NSI results is consistent with loss of large amount of proteins due to freezing, indicting that not only MHC but also many other protein constituents of goat meat were insolubilized during freezing storage. The fact that SDS-PAGE results show no significant difference between fresh and frozen samples is probably related to extraction method used for preparation of samples for electrophoresis. In this procedure ~50 µg protein was loaded into each well; therefore the concentration of protein in each well is similar. It has been suggested that a distinction be made between extarctability and solubility of myofibrillar proteins. While most myofibrillar and sarcoplasmic proteins were soluble at physiological ionic strength and neutal pH, their extractability was not comparable (Hultin, Feng & Stanely, 1995). It is therefore suggested that goat meat might be as soluble as those of other species but its extractability is

different. Taken together, the results of this study indicate that goat meat, like meat of other domestic animals, experiences extensive alteration in proteins when subjected to prolong frozen storage. For prevention of such drastic effects, it is suggested that cryoproctants such as polyoles (sorbitole, sucrose, polydextrins, etc) or polyphosphates be applied to meat prior to freezing (Strasburg, et al, 2008). This information can be useful in developing strategies for storing goat meat and formulation of products made from meat of this species.

IV. ACKNOWLEDGEMENT

This study was financially supported by grants nr 88-GR-VT-11 and from Shiraz Univ. Research Council.

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Figure 1: Effect of storing at -18°C on protein solubility of goat meat. Each value is the mean ± SD

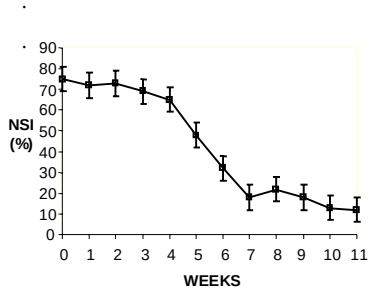


Figure 2: Effect of storing at -18°C on water holding capacity of goat meat. Each value is the mean ± SD

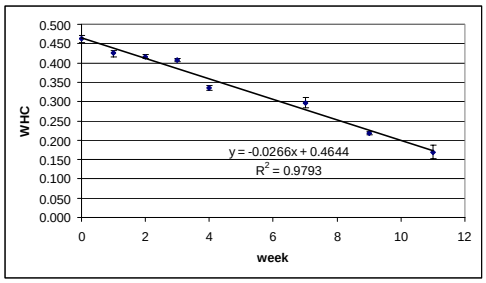
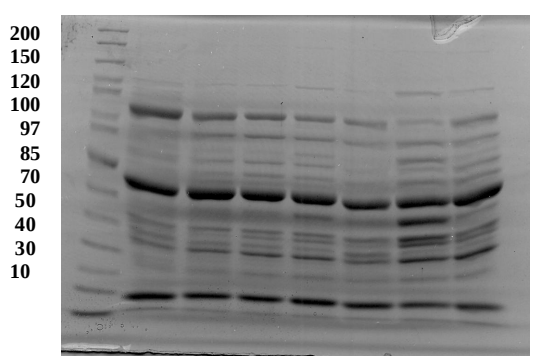


Figure.3: SDS-PAGE of proteins of goat meat stored at at -18°C for different length of time



1) Molecular weight markers, 2) Fresh meat, 3) one week, 4) two weeks, 5) Four weeks, 6) seven weeks, 7) Nine weeks, 8) Eleven weeks.

Table 1: Effect of storing at -18°C on protein solubility and water holding capacity of goat meat. Each value is the mean ± SD. Values with different superscript letters are significantly different (P<0.05). ND: Not Determined

Storage time (week)	WHC (%)	NSI (%)
Fresh	0.46±0.01^{f}	75.65±4.33^{g}
1	0.45±0.01^{f}	72.55±1.66$^{f g}$
2	0.44±0.03^{f}	73.44±2.70$^{f g}$
3	0.39±0.01^{e}	68.84±1.24$^{e f}$
4	0.32±0.01^{d}	65.72±1.93^{e}
5	ND	48.50±3.47^{d}
6	ND	32.26±1.96^{c}
7	0.26±0.02^{c}	17.95±1.53$^{a b}$
8	ND	22.28±9.19^{b}
9	0.21±0.01^{b}	18.32±5.02$^{a b}$
10	ND	13.21±0.92^{a}
11	0.16±0.03^{a}	11.92±0.44^{a}

Table2 : Effect of storing at -18°C on colorimetric parameters of goat meat. Each value is the mean ± SD. Values with different superscript letters are significantly different (P<0.05).

Storage time(days)	**L**	**a**	**b**
0	28±.58^{a}	16.33±4^{a}	12±2.31^{a}
7	26±2.65^{a}	26.67±5.29^{b}	17.67±3.05^{b}
14	29.67±2^{a}	25.33±4.16^{b}	16.33±2.65^{ab}
21	42±4.36^{b}	21.33±6.51^{ab}	15.67±2.08^{ab}