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DETERMINATION OF DIFFERENCES IN FREE AND BOUND WATER CONTENTS OF BEEF MUSCLE BY DSC UNDER VARIOUS FREEZING CONDITIONS

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Abstract

The differences in bound water content of beef semimembranous muscle samples obtained from previously chilled (24 h at +4°C) middle-aged beef carcasses were determined by the use of DSC. Initially, samples obtained from fresh, unprocessed meat were frozen at −40, −50 or −65°C to determine their melting peaks for freezable water (free water) content with the use of DSC. The samples were then subjected to an environment with an ambient temperature of −30, −35, −40 or −45°C, with no air circulation, or with an air circulation speed of 2 m s^{-1}, until a thermal core temperature of −18°C was attained; this was followed by thawing the samples until a thermal core temperature of 0°C was reached. This process was followed by subjecting the samples to the ambient temperatures mentioned above, to accomplish complete freezing and thawing of the samples, with DSC, and thereby determination of the freezable water contents, which were then used to determine the peaks of melting. The calculated peak areas were divided by the latent heat of melting for pure water, to determine the freezable water contents of the samples. The percentage freezable water content of each sample was determined by dividing its freezable water content by its total water content; and the bound water content of each sample was determined by subtracting the percentage free water content from the total. In view of the fact that the free water content of a sample is completely in the frozen phase at temperatures of −40°C and below, the calculations of free and bound water contents of the samples were based on the averages of values obtained at three different temperatures.

Keywords: bound water, DSC, free water

Introduction

This study was based on a previous investigation where the freezable water content of beef semimembranous muscle was determined at different temperatures, together with information regarding what constituents afford the total water content of a sample. Additionally, numerous techniques were used to de-

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termine the free and bound water contents of beef samples, especially DTA and DSC, as discussed previously [1].

Food products are subjected to freezing processes to ensure proper storage; however, these processes result in the free water content of the products being converted into ice crystals, which causes the water activity (a_w) to decrease. This then results in decreases in the chemical and biochemical reaction rates, and slower microbial growth or even a total inhibition of microbial growth [2–4]. The formation of ice crystals and the resultant cell damage are the primary effects of freezing on food quality. Nakayama and Yamamoto [5] established that freezing has undesirable effects on the following properties of meat; pigmentation, aroma and nutritional value, these being affected by the rate of freezing [6, 7]. Numerous researchers have studied the effect of slow *vs.* rapid freezing on the protein denaturation of food products, and have concluded that slow freezing has the greatest effect on the protein denaturation of meat products [8–14], and that the denaturation processes occur especially in myosin, a myofibrillar protein, but less so in actin. Using different techniques, Parducci and Ducworth [15], Heinevetter *et al.* [16] and Wang and Colbe [17] have shown the existence of denaturation processes in the proteins extracted from previously frozen and then thawed meat samples and other food products.

The denaturing of myofibrillar proteins, especially in emulsified products, which are the primary agents for the retention and binding of water, is not desirable in meat products, with the exception of dehydrated meat products, such as pastirma (a special dried Turkish meat product) and sucuk [18, 19]. Therefore, meat used in emulsified products must be rapidly frozen and stored in the frozen state prior to being ground, and it must be introduced directly to the products [20]. Froning and Neelekanton [21] and Lyon *et al.* [22] found that frozen pre-rigor muscles have a higher emulsion capacity and stability than post-rigor muscles.

In the light of the above findings, the main purpose of the present research was to determine the differences caused in the free and bound water contents of meat samples by subjecting the samples to different freezing temperatures and air circulation rates, so as to obtain variable rates of freezing; and to apply these findings to establish the effects on the level of protein denaturation in the samples.

Materials and methods

The source of the meat samples used in this research was the same as previously [1]. The meat consisted of the semimembranous muscle of middle-aged beef carcasses, stored for 24 h at +4°C. All visible fat and connective tissue were removed from the semimembranous muscle prior to its being ground through a 3 mm disc plate.

Prior to DSC study, the samples were frozen in a deep freezer (So-Low, Cincinnati, Ohio, USA) with inside dimensions of 50×200×50 cm, capable of freezing to −85°C, and calibrated to an accuracy of ±0.5°C. The ground beef samples

were placed in containers made of pure copper, measuring 8.6 cm in height, 7.0 cm in width, and 4.0 cm in depth. An opening large enough to insert a thermocouple was created in the center of the samples, and the thermocouple was placed in this opening to measure the temperature variations in the thermal core of each sample. Each sample container was wrapped in aluminium foil to prevent loss of water from the samples during the freezing and thawing processes, which could be caused by the open space above and below the sample. In order to accomplish a uniform initial temperature throughout the sample, sample containers were stored in a refrigerator (4°C) for a 12 h period. Following refrigeration, samples were placed in a freezer with an ambient temperature of −30, −35, −40 or −45°C and an air circulation rate of 0 or 2 m s^{-1} until the thermal core temperature of each sample reached −18°C. The frozen samples were then placed in a commercial grade refrigerator, where an ambient temperature of 7°C and an air circulation rate of 2 m s^{-1} were applied to each sample. The samples were stored in the refrigerator until the thermal core temperature of 0°C was reached. As outlined previously [1], DSC was used to freeze the samples again at −40, −50 or −65°C, and then to thaw to a temperature of 35°C. The freezing of the free water content of beef samples is complete at temperatures of −40°C or below [23–28]. The free and bound water contents of the samples were calculated by the method of Aktas *et al.* [1], by taking the averages of the DSC results where the three different temperatures were used.

Results and discussion

The water contents of the samples were classified into two categories; free and bound water, based on a temperature of −40°C. This is the reference temperature, accepted by numerous researchers [23–28], where the water content that achieves complete freezing is termed free water; and that remaining unfrozen is termed bound water. The total, free and bound water contents of the samples, as determined by DSC, at different ambient temperatures and air circulation rates, are shown in Table 1. Initial temperatures and latent heats of thawing are presented in Table 2.

The highest total water content was 76.27%, and the lowest was 74.83%; an increase in the total water content of a sample resulted in an increase in the freezable (free) water content and a decrease in the bound water content, as shown in Table 1. As examples, the freezable and bound water contents of a sample frozen under an ambient temperature of −35°C and an air circulation rate of 2 m s^{-1}, which has the lowest total water content, and of a sample frozen under an ambient temperature of −40°C and an air circulation rate of 0 m s^{-1}, were determined to be 69.02%, 30.98%, 71.04% and 28.96%, respectively. The sample with the highest total water contained 74.78% free and 25.22% bound water. Similar results were obtained by Riedel [23], Chen [25], Pham [27] and Bartlett [29] for lean beef, cod fish, and orange juice, as shown in Table 3.

Table 1 The determined free and bound water contents of beef, previously frozen and thawed under various temperatures and rates, re-frozen to –40°C with DSC

Ambient temperature during freezing/°C	Air circulation rate/m s^{-1}	Total water	Free water	Bound water
		content/%		
–30	0	75.21	71.72	28.28
	2	75.06	69.78	30.22
–35	0	74.97	73.65	26.35
	2	74.83	69.02	30.98
–40	0	74.83	71.04	28.96
	2	75.52	71.26	28.74
–45	0	76.09	73.77	26.23
	2	76.27	74.78	25.22
Unprocessed meat (fresh meat)		75.03	68.98	31.02

Table 2 Initial temperature of melting and latent heat of melting as determined with DSC for beef frozen under variable temperatures and air circulation rates

Ambient temperature during fereezing/°C	Air circulation rate/m s^{-1}	Initial temperature of melting/°C	Latent heat of melting/J g^{-1}
–30	0	–3.03	189.18
	2	–2.97	183.56
–35	0	–2.90	193.52
	2	–3.01	180.98
–40	0	–3.08	186.30
	2	–3.01	188.59
–45	0	–2.65	196.72
	2	–2.76	199.90
Unprocessed meat (fresh meat)		–2.75	181.38

The fact that an increase in total water content is accompanied by an increase in freezable water content and a decrease in bound water content may be due to a decrease in the ratio of the water binding components of the samples. Pham [27] postulated that a decrease in solid matter content of a sample results in a decrease in the water binding protein molecules, which in turn causes a decrease in the bound water content of a given food material. Chen [25] additionally demon-

Table 3 Calculated *vs.* experimental bound water content at –40°C

Food items	Solid content/ %	Water content/ %	Bound water content at –40°C/%				
			Riedel [23] (Expt)	Schwartzberg [24]	Bartlett [29]	Chen [25]	Pham [27]
Lean	26	74	12	8.8	2.0	10.8	10.2
beef	50	50	36	25.0	5.4	38.9	40.9
Cod	18	82	8	5.5	1.8	7.5	6.5
fish	50	50	39	25.0	7.5	41.9	38.6
Orange	11	89	2	1.9–3.7	2.4	2.1	–
juice	50	50	17	15–30	24.3	24.9	–

strated that the initial point of freezing has a substantial effect on the bound water content of any food material. The present and the previous study [1] reveal that the latent heats of thawing are 12% lower than the differences in the total enthalpy. Taking this 12% difference into account results in a freezable water content of 80.98%, while the bound water content becomes 19.02%. When this 19.02% bound water content is compared with the bound water contents listed in Table 3, it is apparent that the value of 19.02% is high. However, it is also clear that studies on determination of the bound water content of any food material must continue until the fastest and most sensitive techniques are fully developed and routinely used, as evidenced by the discrepancy in the findings of different researchers on the same food samples.

Table 4 Freezing times and freezing rates required for meat samples to be frozen under various ambient temperatures and air circulation rates

Ambient temperature during freezing/°C	Air circulation rate/m s^{-1}	Freezing time/ min	Freezing rate/ cm h^{-1}
–30	0	244	0.491
	2	46	2.608
–35	0	213	0.563
	2	39	3.076
–40	0	190	0.631
	2	35	3.428
–45	0	161	0.745
	2	30	4.000

Results on the freezing time and rate of freezing required in the freezing process of meat samples under ambient temperatures of –30, –35, –40 or –45°C at air circulation rates of 0 or 2 m s^{-1} are presented in Table 4.

As can be seen from Table 4, eight different freezing rates were applied to the meat samples. Polymenidis [30], Wirth [31] and Gökalp and Tülek [32] classify freezing at a rate of 0.2–1 cm h^{-1} as slow freezing; at a rate of 1–5 cm h^{-1} as quick freezing; and at rates greater than 5.0 cm h^{-1} as shock freezing. On this basis, the freezing rates applied in this study can be classified as slow or fast freezing.

As can be seen from Table 1, both the slow and quick freezing processes caused an increase in the free water content and a decrease in the bound water content as compared to fresh meat. The free and bound water contents of fresh (unprocessed) meat were 68.98% and 31.02%, respectively, whereas the average free and bound water contents of meat subjected to the 4 slow and 4 quick freezing processes were 71.21, 28.79, 72.55 and 27.45%, respectively. These results show that the 31.02% bound water normally present in beef decreases by 3.57 and 2.23% under slow and quick freezing, respectively. This decrease in the ratio of bound water was 11.50 and 7.18% under slow and quick freezing, respectively. The cause of the decrease in the bound water and the increase in the free water content is thought to be partial denaturation of the protein compositions during the freezing and thawing processes. Wagner and Anon [7] concluded from their DSC studies that various freezing rates cause various degrees of denaturation of myofibrillar proteins. Triple endotherms have been obtained for myofibrillar proteins of fresh and frozen beef muscle samples which have had their sarcoplasmic and connective tissue proteins removed. The triple endotherms were evaluated with the following results: the secondary and tertiary endotherm areas remained constant, while the primary endotherm area, in conjuction with the total endotherm area, decreased. This decrease was greater in samples subjected to a slow freezing process, followed by thawing, than those observed for medium and quick freezing rates, as seen in Table 5.

The resultant drop in the endotherm areas is directly proportional to the rate of denaturation of the proteins. This is due to the areas of myofibrillar proteins

Table 5 The effect of specific freezing rates on DSC determined peak areas of myofibrillar proteins isolated from beef [Wagner and Anon [7]]

Rate of freezing/ min s^{-1}	Specific area/cm^2 mg^{-1}			
	Total endotherm	Peak 1	Peak 2	Peak 3
Fresh meat	5.78±0.27	2.20±0.22	1.42±0.27	2.16±0.16
High (t_c<5)	3.13±0.19	1.77±0.14	1.40±0.20	1.96±0.11
Medium ((t_c=20–25)	4.81±0.28	1.59±0.17	1.37±0.11	1.85±0.12
Low (t_c>60)	4.87±0.28	1.50±0.15	1.46±0.16	1.91±0.11

Table 6 Denaturation enthalpies of myofibrillar proteins isolated from fresh and frozen beef [Wagner and Anon [7]]

Freezing rate of beef muscle myofibrillar/min	ΔH_d/ (cal g^{-1} dry matter)	Loss of enthalpy/% Compared to fresh meat
Fresh	5.51±0.10	–
High (t_c<5)	5.33±0.06	3.27
Medium (t_c=20–25)	5.06±0.05	8.17
Low (t_c>60)	5.01±0.05	9.07

isolated from previously frozen and thawed meat having lower enthalpies than those for proteins in their natural state.

Wagner and Anon [7] found that freezing led not only to denaturating of the protein molecule, but also to partial unfolding of the molecule, which results in the formation of hydrophobic groups. They concluded that this partial unfolding of the molecules may have caused an increase in the ionic intensity and the separation of water from the surface of the myofibrillar proteins, or dehydration of the myofibrillar proteins. This phenomenon was especially apparent at low freezing rates. Their findings show a parallelism with the present findings. In other words, from the decrease of 11.50% in the bound water ratio for the slow freezing process, as compared to the decrease of 7.18% for the quick freezing process, it is evident that in the slow freezing process the denaturation of the proteins is greater.

It is preferred that high-quality meat should contain a high percentage of bound water content, with the exception of meats used for dried or semi-dried meat products such as pastirma and sucuk. Therefore, it may be postulated that the use of previously frozen and then thawed meats in all applications except those noted above may be disadvantageous as concerns the quality of the resultant meat product.

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