Fat-Holding in Hamburgers as Influenced by the Different Constituents of Beef Adipose Tissue

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FAT-HOLDING IN HAMBURGERS AS INFLUENCED BY THE DIFFERENT CONSTITUENTS OF BEEF ADIPOSE TISSUE

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Abstract

The effects of addition of adipose tissue, rendered fat, rendered fat + the separated connective tissue and rendered fat + gelatin, respectively, were compared on the fat-holding properties of hamburgers upon heating.

The fat losses on frying (175 °C, 3.5 minutes on each side) were substantially less than in the net test (cooking in a water bath at 77 °C for 35 minutes, followed by a centrifugation step). Fat losses during frying were governed both by the instability of the fat and the migration of the fat out of the product. The fat losses determined by the net test, however, reflected only the instability of the fat, as the effect of the migration of the fat out of the product was minimized, due to the centrifugation step.

Fat-holding on frying was the best when fat was added in the form of fat cells and when an increased amount of connective tissue was added. On the contrary, fat-holding in the net test was best, when the fat was emulsified to smaller droplets with gelatin as an emulsifier. These observations suggest that the migration of the fat out of the hamburger is the most important factor, governing fat-holding in hamburgers on frying, whereas for the net test the instability of the fat *per se* seemed to be more essential.

Introduction

One of the most important features in the production of comminuted meat products is the achievement of high cooking stability, i.e., to prevent fat, as well as water, separating from the product on heating.

In a comminuted meat batter, the fat is dispersed in a protein matrix. The fat can exist in aggregates of fat cells, single fat cells or can be squeezed out of the fat cells, forming small droplets, larger fat pools or fat channels. The fat cells are held in a matrix in the adipose tissue, consisting mainly of collagen, which can be converted to gelatin on heating. The proteinaceous part of the adipose tissue is seldom taken into account, rather the lipid part, when discussing fat-holding in meat products. Evans and Ranken (1975), however, found that the connective tissue could influence the fat loss on cooking. In their investigation, fat losses increased with greater hardness of the fat, and they stated that it was mainly due to the connective tissue content, rather than to the softness of the lipids. In a later investigation by Tornberg and Persson (1987), the fat-holding properties of the adipose tissue itself, from pork and beef, have been compared on heating. They have suggested that the contraction of the connective tissue is more severe for beef than for pork adipose tissue on heating, thereby giving rise to a lower fat-holding for the former, compared to the latter.

With regard to fat-holding, there is an extensive literature on the emulsifying properties of meat proteins. Studies have been conducted in model systems using oil-in-water emulsions prepared with protein solutions and oil (Hegarty *et al.*, 1963; Acton and Saffle, 1970, 1972; Ivey *et al.*, 1970; Gillett *et al.*, 1977; Li-Chan *et al.*; 1984). The emulsifying properties of the proteins have, however, been considered to be less important than generally assumed. In solid and semi-solid food products, where fat is dispersed in a continuous matrix, fat-holding properties are influenced by other factors in addition to the interfacial film surrounding the droplets, and the whole structure of the meat product has to be taken into account (Hermansson, 1986).

The importance of the protein matrix for the fat-holding properties in finely comminuted meat systems

has been observed by several research workers (Lee *et al.*, 1981; Lee, 1985; Acton *et al.*, 1983; Jones, 1984; Comer *et al.*, 1986; Tornberg *et al.*, 1989). The literature, however, mainly concerns finely comminuted meat systems, i.e., sausages (Ackerman *et al.*, 1971; Brown and Toledo, 1975; Carroll and Lee, 1981).

However, the mechanism of fat-holding is not the same in every type of meat product. Tornberg *et al.* (1989) have shown that fat release during the frying of hamburgers, i.e., a less comminuted meat product, is mostly affected by an increase in the fat content, whereas this relationship does not hold for fat release during the frying of cooked sausages. For the latter products, the fat loss correlates better with the water loss. The mechanisms behind the two different behaviors in these two meat products are suggested as follows: for hamburgers the probability of encounter between fat droplets seems to be the most dominant factor in controlling fat release during frying, whereas the protein matrix is more important for fat-holding during the frying of cooked sausages.

The common opinion has been that the myofibrillar and sarcoplasmic proteins, mainly myosin and/or actomyosin, are responsible for the fat-holding properties of comminuted meat products by forming an interfacial film around the fat droplets. Many research workers, however, stress the importance of the gel-forming properties of the meat proteins, especially myosin (Hermansson *et al.*, 1986; Egelandsdal *et al.*, 1986; Wicker *et al.*, 1986; Siegel and Schmidt, 1979; Ishioroshi *et al.*, 1979). Moreover, recent investigations show that collagen can be an integral part of the meat protein matrix (Hermansson, 1988).

Recent results from our laboratory suggested that the fat-holding properties of comminuted meat products can be influenced by the formation of gelatin upon cooking (unpublished results). Gelatin can be formed from collagen on heating, where the collagen exists both in the muscle and in the adipose tissue. The aim of this investigation was, therefore, to study how the different constituents of beef adipose tissue influence the fat-holding properties of a coarse-ground meat system (hamburger patties) on heating, under two different heating conditions, namely, fried at 175 °C for 3.5 minutes on each side and cooked in a water bath at 77 °C for 35 minutes. Fat losses were measured as a function of fat content (14-26%), as this parameter explains most of the variation in fat-holding (Cross *et al.*, 1980; Tornberg *et al.*, 1989).

Materials and Methods

Raw Materials

The raw material was obtained from a nearby slaughterhouse (Skanek, Kävlinge), three days after slaughter. The beef fat samples was taken from the groin area of young bulls, while the beef muscle was *M. biceps femoris* taken from young bulls. The gelatin used (220 bloom) was purchased from Extraco, Sweden.

Rendered fat from the adipose tissue

The adipose tissue was ground twice through a 3 mm plate. It was set in a boiling water bath and heated until the temperature of the fat had reached 80 °C. It was then transferred into an 80 °C oven, where the fat was filtered off through filter papers. The rendered fat was stored at 4 °C until being used.

Separation of connective tissue from the adipose tissue

The beef fat was cut into pieces approximately 5-10 cm in size. The fat was ground through a 3 mm plate and further comminuted into about 200 g portions in a mixer (Robot Coupe 3000, Robot Coupe S.A., France) for about 30 seconds. The comminuted fat was then transferred into a 50 °C oven for about two hours, where part of the fat melted into Erlenmeyer flasks, while the remaining portion was collected into filters.

The fat still adhering to the connective tissue was removed by extraction with chloroform: samples of the partly-defatted adipose tissue (approximately 50-60 g) were put into a mortar and 40 ml portions of chloroform were added. Each portion of chloroform was thoroughly mixed with the sample and the mixture was filtered using suction. The latter procedure was repeated four to five times.

Preparation of hamburger patties

Hamburger patties were prepared using beef muscle, beef fat, salt and iced water. The fat was added in the form of beef adipose tissue, rendered fat, rendered fat + the same amount of separated connective tissue as in the adipose tissue, rendered fat + three times the amount of separated connective tissue as in the adipose tissue, and rendered fat + gelatin corresponding to the same amount of collagen as in the adipose tissue.

The patties were prepared by mixing 70% ground meat, 16-24% fat, 1.6% NaCl of the total weight and water. As the fat content was raised, the addition of water was lowered, i.e., the protein content was kept constant.

Pieces of meat and fat, approximately 5-10 cm large, were ground together with the connective tissue through a 3 mm plate and mixed with NaCl and water for 2 minutes in a Hobart mixer (model PF 401) at a low speed. In the case of gelatin added, the gelatin was dissolved in part of the water by heating to about 60 °C. The water solution of gelatin, was added after cooling to about 40 °C. A Hollymatic machine (model 54) was

used to produce patties (100 mm in diameter, 10 mm thick and approximately 80 g in weight). The resulting patties were frozen at -25 °C and stored at the same temperature until analysis (after approximately one week).

Chemical analysis

The contents of water, fat, protein and hydroxyproline were analyzed for some of the raw materials and all hamburger patties, in accordance with the procedure described by Fjelkner-Modig and Tornberg (1986). The contents of water and fat was also analyzed in the cooked hamburger patties. The collagenous connective tissue content was calculated by multiplying the hydroxyproline content by a factor of 8 (Kolar, 1990).

Light Microscopy

Samples were taken from the hamburgers, raw and cooked. Sample blocks (15 x 5 x 5 mm) were frozen in liquid nitrogen, if the samples were not previously frozen. This was the case for the raw hamburgers, frozen at -25 °C.

The samples were cryo-sectioned using a Leitz Cryostat 1720 Digital. The temperature of the cryostat chamber was maintained at -20 °C. The sections, 8-10 μm thick, were mounted on gelatin-coated microscope slides. Nile blue (0.4% water solution) for 2 minutes was used for staining some of the sections. They were subsequently rinsed with distilled water and covered with cover glasses. After staining, the fat became pink and the protein blue. The Nile blue-stained sections were exposed to UV-light, which made the fat fluoresce in a yellow color, whereas other components did not. Sections were also stained with aniline blue and orange G (0.10% orange G for 5 minutes followed by rinsing in distilled water for 1 minute and 0.07% aniline blue for 4 minutes followed by rinsing in distilled water for 5 minutes). Using this technique the collagen/gelatin became blue, and the myofibrillar proteins yellow, while the fat was unstained.

The sections were examined and photographs were taken under a light microscope (Nikon Optiphot) at a magnification of 134x.

Image analysis

The photographs were evaluated using an image analyzing system LABEYE/3PC (Innovativ Vision AB, Sweden) to calculate the fat droplet size distribution. For the size distribution analysis, we have used a surface/length average of the fat droplet size, d_{al}, as described by Tornberg et al., 1989.

Fat losses

The net test, according to Hermansson and Luciano (1981), was used to determine the fat-holding properties of the hamburger patties by heating 10 g samples (n =

7) in a water bath at 77 °C for 35 minutes. The samples were centrifuged for 20 minutes at 500 g after heat treatment. The fat loss during frying (175 °C, 3.5 minutes on each side; a center temperature of about 70 °C) was also determined (n = 6). The fat loss was expressed as the percentage fat loss based on the fat content of uncooked product.

Statistical analysis

The statistical analysis was performed using SYSTAT (The system for statistics), SYSTAT, Inc, Evanston, Illinois. The programs used were linear regression, t-test and covariance analysis.

Results

Description and chemical composition of the different parts of the adipose tissue added

As shown in Table 1, 68% of the protein in the adipose tissue was collagen. The rendered fat contained virtually any proteinaceous matter. In the separated connective tissue there was some fat left, but the protein and collagen contents were raised from 4.0 to 31.7% and from 2.7 to 21.4%, respectively.

The purpose of this work was to find out how the different constituents of the adipose tissue influence the fat-holding properties of hamburgers upon heating. Therefore, the content and type of lean meat and salt was kept constant, while the fat was added in different ways. Moreover, the fat content was varied between 16-24%, since this factor has been found to be one of the most important factors regarding the fat-holding of hamburger patties (Tornberg et al., 1989). The chemical compositions of the different hamburgers are shown in Table 2.

The first type of hamburger patty (A) was made with the adipose tissue fat added, when the fat consisted of fat cells, fat cell aggregates, fat exuded out of the fat cells in the form of fat pools or small droplets, connective tissue and gelatin, i.e., a rather complex system.

In type B, rendered fat was used instead of adipose tissue. This can clearly be seen in Table 2, showing that the collagen content of the type B hamburgers was 1.1% on average, while the collagen content of the type A hamburger was 1.5%. Therefore the fat in hamburgers of type B was only present in the form of fat pools and droplets.

The third type of hamburger (C) was prepared with rendered fat and connective tissue, in the same amount as type A. It can also be seen in Table 2 that the collagen content of A and C is about the same. The difference between the two types of hamburger, is that the fat in the hamburger type C was in the form of fat pools and fat droplets while that in the hamburger type A

Table 1. Chemical composition of the beef adipose tissue, the rendered fat and the separated connective tissue expressed as the mean average (x̄) and the standard deviation (sd).

Sample	n	Fat (%)		Water (%)		Protein (%)		Collagen (%)	
		x̄	sd	x̄	sd	x̄	sd	x̄	sd
Beef adipose tissue	8	80.8	4.3	14.7	3.0	4.0	1.2	2.7	0.7
Rendered fat	3	99.2	1.1	0.2	0.0	0.04	0.05	0.0	0.0
Separated connective tissue	5	3.0	1.4	68.2	3.1	31.7	8.5	21.4	4.5

Table 2. Chemical composition of the raw hamburgers and collagen content of the liquid phase separated using the net test, expressed as a average (x̄) and standard deviation (sd).

Hamburger	Type	n	Fat (%)		Water (%)		Protein (%)		Collagen (%)		Collagen in liquid phase from net test (%)	
			x̄	sd	x̄	sd	x̄	sd	x̄	sd	x̄	sd
Adipose tissue	A	10	18.6	4.2	63.0	4.4	15.5	0.4	1.5	0.2	0.2	0.1
Rendered fat	B	10	18.9	3.4	63.8	3.4	15.2	0.3	1.1	0.1	0.2	0.1
Rendered fat + connective tissue	C	10	19.3	4.8	62.7	4.2	15.8	0.6	1.5	0.2	0.2	0.1
Rendered fat + 3x connective tissue	D	5	18.0	2.7	63.5	3.2	17.1	0.7	2.7	0.5	0.5	0.2
Rendered fat + gelatin	E	5	18.7	3.9	62.3	3.9	16.5	0.3	1.9	0.3	4.4	1.2

existed in the form of fat cells.

In type D, rendered fat was added together with three times the amount of separated connective tissue, as in A and C. The collagen content of these hamburgers therefore increased by about 2.7%, while the fat content was the same as the others.

Finally, type E was prepared with rendered fat and gelatin in the same amount of collagen as types A and C.

Fat losses in hamburgers on frying and cooking

Fat separation in meat products during cooking is mainly dependent on two factors: The instability of the fat and the possibility to migrate the fat from the inner to the outer parts of the product (Tornberg et al., 1989). The instability of the fat itself and the migration of the fat out of the product were differentiated by using frying and the net test where the net test did not count the migration of the fat, since it included a centrifugation step after heating.

When measuring the fat loss upon frying, not only the instability of the fat itself has to be taken into account, but also the influence of the protein network must be considered since no external force has been applied to the hamburgers in this type of test.

In Figure 1 (left), the fat losses on frying as a function of fat content, for the different types of hamburger patties made (A-E), can be compared. In the same figure (right), the fat losses as determined by the net test, are shown for the same hamburger patties.

The fat losses on frying in the hamburger patties made with the adipose tissue fat added were low at small fat contents and increased linearly with the fat content (r = 0.98***) (Figure 1A, left). This was in agreement with earlier studies (Tornberg et al., 1989). The corresponding relationship for the fat losses determined by the net test was also linear (r = 0.88***), but the fat losses were significantly higher (p < 0.001) (about 30%). The higher value in the fat losses in the net test can be

Figure 1. Fat loss (%) on frying (left) and as determined using the net test (right) as a function of fat content (%) for hamburger patties with **A**) adipose tissue, **B**) rendered fat, **C**) rendered fat + separated connective tissue, **D**) rendered fat + three times the amount of separated connective tissue, and **E**) rendered fat + gelatin.

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due to the centrifugation step follow cooking, which forced the melted fat out of the product. Furthermore, the longer cooking time when compared to frying, allowed the fat to coalesce more and therefore more easily to separate out of the product.

Tornberg *et al.*, (1989) found that in hamburgers having a fat content of 20%, 60-70% of the fat is unstable, i.e., exists in ruptured fat cells or in fat pools, as estimated by measuring the percentage of fat extracted by hexane, according to the method of Tinbergen and Olsman (1979) and modified by Tornberg and Ediriweera (1988). This value is about the same as that measured by the net test at the same fat content (see Figure 1A, right), suggesting that most of the unstable fat is released from the product during centrifugation in the net test.

When only rendered fat was used instead of fat adipose tissue, fat losses as a function of fat content on frying and cooking are shown in Figure 1B. Linear, but insignificant relationships were obtained, when the frying test ($r = 0.44^{n.s.}$) and the net test ($r = 0.50^{n.s.}$) were used. It can further be observed that fat losses were higher both on frying ($p < 0.05$) and cooking (n.s.) for the hamburgers with rendered fat, when compared to hamburgers with adipose tissue.

The addition of separated connective tissue did not improve the fat-holding properties of the hamburgers, neither on frying nor on cooking, as seen in Figure 1C. The correlation coefficients between the fat losses on frying and cooking and fat content were $r = 0.75$ ($p < 0.05$) and $r = 0.56$ (n.s.), respectively.

When the amount of connective tissue added to the hamburger patties was tripled, the fat losses on frying, however, decreased significantly ($p < 0.001$) (Figure 1D, left), when compared to the hamburgers with rendered fat, shown in Figure 1B, left. On the contrary, the fat-holding ability, of the same hamburgers, as determined by the net test, was the poorest. The fat loss as determined by the net test was higher ($p < 0.001$) than that on frying for the type D hamburger.

The fat losses in the fried hamburgers prepared with rendered fat and gelatin (E), were highly correlated with the fat content ($r = 0.95^{*}$) being
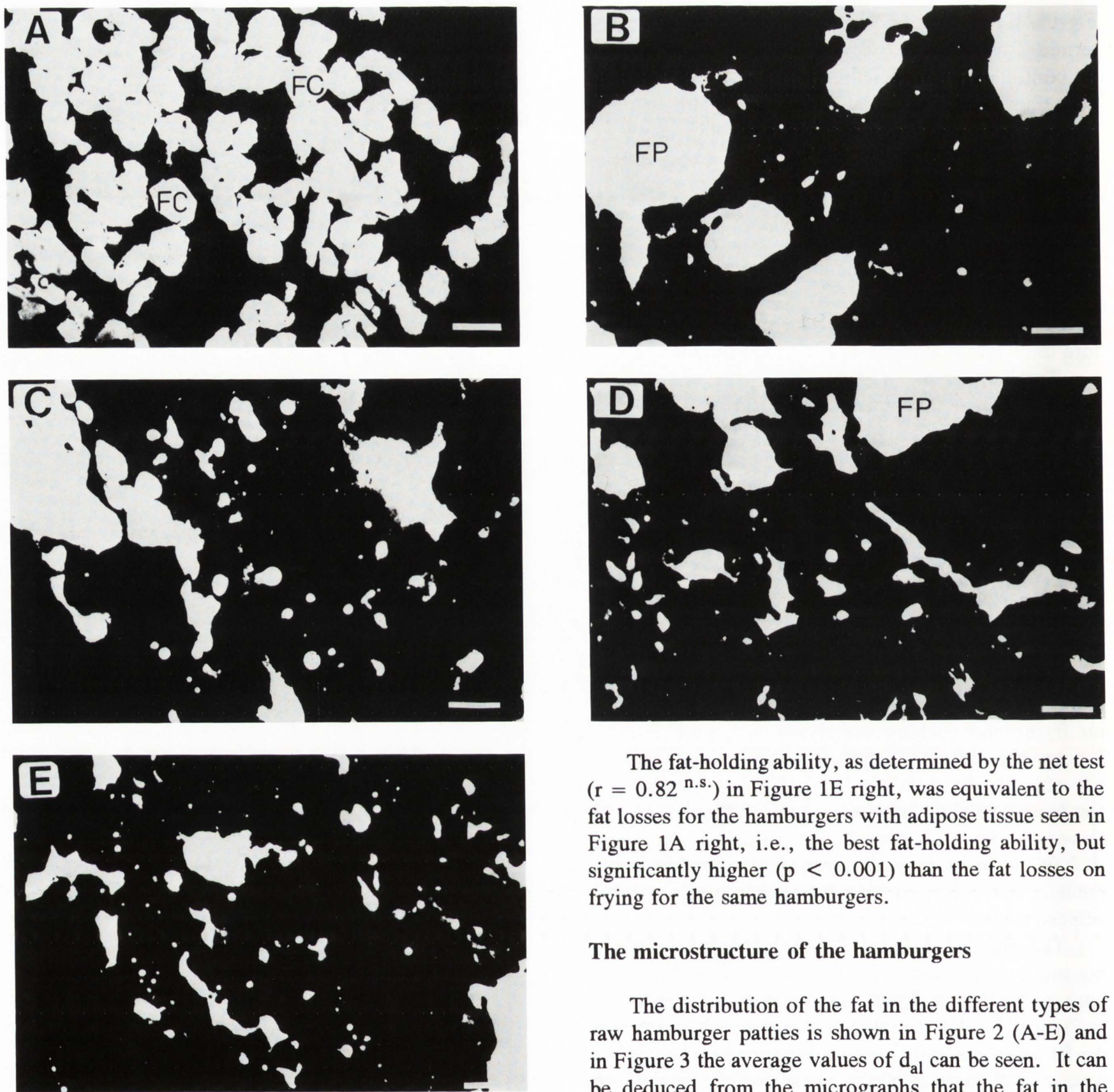

FRYING | THE NET TEST

FAT LOSS (%)

FAT CONTENT (%)
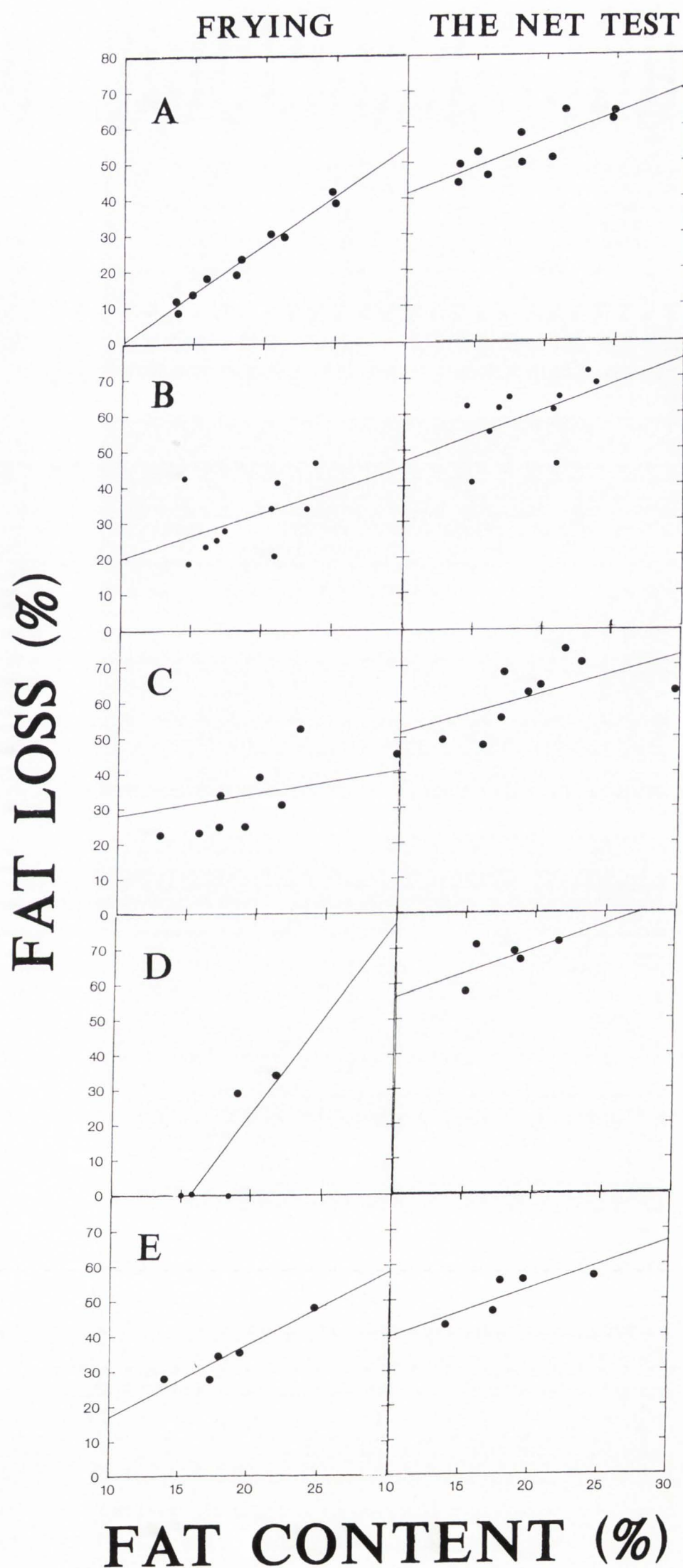
Figure 2. Transverse sections of raw hamburger patties with **A**) adipose tissue, **B**) rendered fat, **C**) rendered fat + separated connective tissue, **D**) rendered fat + three times the amount of separated connective tissue, and **E**) rendered fat + gelatin. The sections were stained with Nile blue and exposed to ultra-violet light. Bar = 100 μm; FC = fat cell; FP = fat pool.

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similar to these in the hamburgers with rendered fat only (B), as seen in Figure 1, left. This means that the gelatin does not significantly influence the fat-holding ability on frying.

The fat-holding ability, as determined by the net test ($r = 0.82$ [n.s.]) in Figure 1E right, was equivalent to the fat losses for the hamburgers with adipose tissue seen in Figure 1A right, i.e., the best fat-holding ability, but significantly higher ($p < 0.001$) than the fat losses on frying for the same hamburgers.

The microstructure of the hamburgers

The distribution of the fat in the different types of raw hamburger patties is shown in Figure 2 (A-E) and in Figure 3 the average values of d_{al} can be seen. It can be deduced from the micrographs that the fat in the hamburgers with adipose tissue (Figure 2A) was mostly in the form of fat cell aggregates and separate fat cells. The size of the fat cells was about 80 μm on average. In contrast, the fat in the hamburger patties with rendered fat (Figure 2B) was in the form of large fat pools and only to a minor extent as small droplets. According to Figure 3, the average size of the fat pools was about 98 μm in type B hamburgers, i.e., larger than in type A hamburgers, as may be clearly seen from the micrographs. The standard deviation of d_{al} was also higher for the type B hamburgers, indicating a large variation in the size of the fat ranging from small droplets to large fat pools in those types of hamburger.
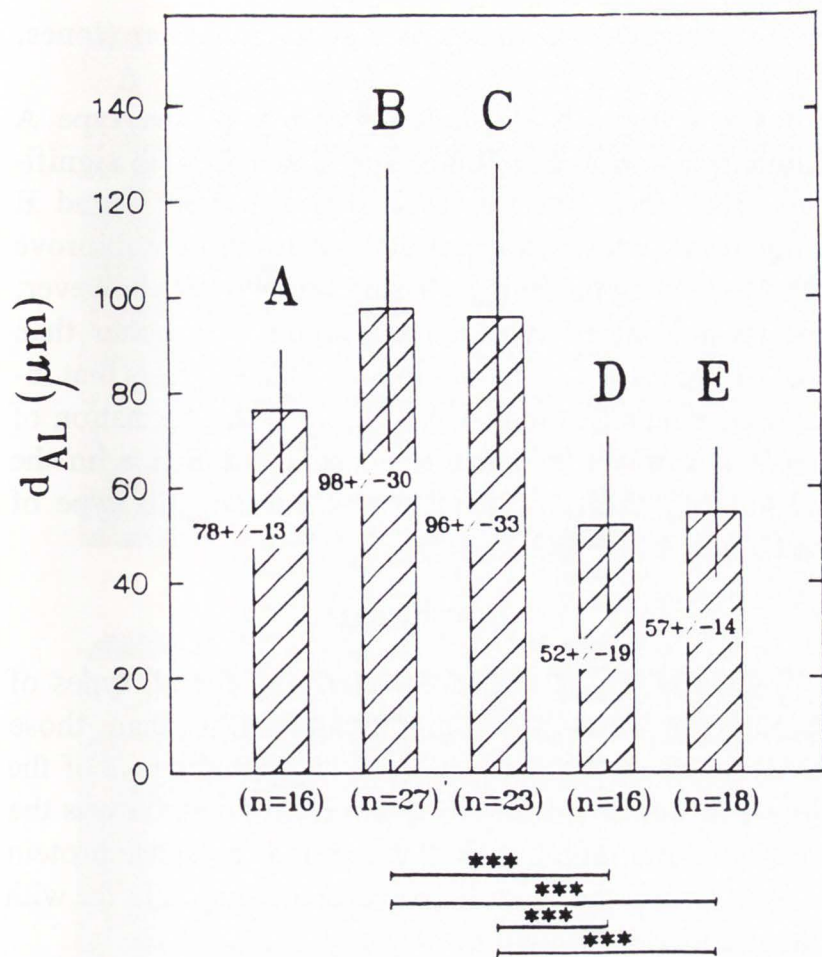
Figure 3. Characteristics of fat droplet size distribution of hamburger patties with **A**) adipose tissue, **B**) rendered fat, **C**) rendered fat + separated connective tissue, **D**) rendered fat + three times the amount of separated connective tissue, and **E**) rendered fat + gelatin. D_{al} (surface/ length average of the droplet size) is given as average and with standard deviation. The lines below the diagram connects the types of hamburger between which there were significant differences.

In Figure 2C the microstructure of the fat in a raw hamburger patty with rendered fat + separated connective tissue, to the same amount as in adipose tissue, can be seen. The distribution of the fat in the hamburger type C was similar to the one in the hamburger type B (Figure 2B) according to Figure 3. This result shows that the addition of connective tissue did not influence the dispersion of the fat.

The fat distribution in the hamburgers with an increased amount of connective tissue (Type D) can be seen in Figure 2D. According to Figure 3, the size of the fat pools was significantly less in this type of hamburger, when compared to types B and C.

The fat distribution in the hamburger patty prepared with gelatin (Type E), is shown in Figure 2E, which contained significantly smaller fat pools, when compared to those prepared with rendered fat only (B) or with rendered fat plus connective tissue (C).

The standard deviations of d_{al} for the hamburger,

type D and E, were smaller than those for types B and C, suggesting that the fat was more evenly distributed in the hamburger types D and E.

In Figure 4, the microstructure of the different types of fried hamburger patty are compared. It can be deduced from Figure 4A that the connective tissue around the fat cells still existed to a certain degree after frying.

In the micrographs of the hamburger patties prepared with rendered fat (Figure 4B), no connective tissue/gelatin network is seen, since the connective tissue from the adipose tissue was removed in this type of hamburger. The collagen/ gelatin seen in the micrographs is therefore assumed to originate from the muscle.

From the micrograph of the hamburger prepared with the connective tissue plus rendered fat more collagen/gelatin is seen, when compared to Figure 4B, but the collagen is not in the form of a network as in Figure 4A. The microstructure of the fried hamburger patty prepared with the addition of three times as much collagen as in A and C, is shown in Figure 4D. As can be seen from the micrograph, collagen/gelatin is located around and between the muscle fibers building up a more dense protein structure than those which can be seen in Figures 4B and 4C. In Figure 4E, representing the structure of the fried hamburger with rendered fat and gelatin, this dense structure can no longer be seen.

Discussion

The results clearly show, that the fat losses, as determined by the net test, were significantly ($p < 0.001$) larger than those on frying, for all types of hamburger investigated (A-E). This is probably due to the centrifugation, used in the net test, where centrifugation forces the fat out of the product and the longer cooking time allows the fat to coalesce more easily. Therefore, the migration of the fat out of the product is not a limiting factor, when using the net test.

This observation that the fat loss is much higher, using the net test as opposed to frying, suggests that the protein network hinders the migration of the fat out of the product, thus helping the fat-holding in the meat product.

Hamburgers made of adipose tissue (A), as opposed to those made of rendered fat (B-E), had good fat-holding properties especially on frying. The microstructure of the fried type A hamburger (Figure 4A) suggests that the collagen network left in the adipose tissue might contribute to the good fat-holding through a high resistance to the transfer of fat from the inner to the outer parts of the product. The relationships between fat loss and fat content for type A hamburgers had higher and more significant correlation coefficients than the relationships

between fat loss and fat content for the hamburgers with rendered fat.

The hamburgers with rendered fat, without (B) and with (C) the addition of separated connective tissue, have larger deviations from linearity between fat loss and fat content and also the widest size distributions of the fat pools. This observation suggests that the dispersion of the fat has some influence on the fat-holding properties of the hamburgers. On frying, the hamburgers with the largest fat pools (B and C) also had the largest fat losses, but this difference was not as pronounced in the net test. This suggests that the migration of the fat was facilitated by larger fat pools and that fat channels transporting the fat from the inner to the outer parts of the products were much more easily formed out of larger fat pools.

Based on the net test, the greatest fat-holding ability was found in the hamburgers made with rendered fat and gelatin (E). These hamburgers had small fat-pools, according to Figure 3. On the other hand, the hamburgers made with rendered fat and an additional amount of connective tissue (D) had similar fat pool sizes, but the highest fat losses.

It was observed during mincing that the addition of connective tissue to type D caused an increased disintegration of the fat. Since the collagen has most likely not been converted to gelatin to any large extent in the hamburgers, the total amount of protein in the water phase of the meat system is less in D than in E. It might be that the fat pools in the former type are covered by less protein molecules than the fat pools in the latter type.

The addition of connective tissue (C) in the same amount as in the hamburgers with adipose tissue (A) did not influence the dispersion of the fat and the fat-holding ability, when compared to hamburgers with rendered fat only (B).

The addition of three times as much connective tissue like in type D hamburger, however, decreased the size of the fat pools and also produced a protein structure in the fried hamburgers that differed from the other types, being more dense. The fat-holding ability of those hamburgers (D) was significantly better (***) than that of C on frying but it was not improved when the net test was used. The greater fat-holding values measured by the frying test than the net test reflected the limited migration of the fat through the dense protein structure, without a centrifugation treatment.

In type A, C and E hamburgers, the same amount of collagen has been added, but in different forms, i.e., as a network around whole fat cells (A), as separated connective tissue (C) and as gelatin (D). The addition of gelatin to hamburgers produces the smallest fat pools. This suggests that the emulsification of the fat has been facilitated by the addition of gelatin, which could be the case since gelatin is known as a good emulsifier (Jones, 1977).

On frying, the fat-holding ability of the type A hamburgers was better than type C and E. No significant difference was observed between type C and E hamburger, which indicates that gelatin did not improve the fat-holding on frying. Using the net test, however, the fat-holding of type E hamburgers was better than that of type A and C hamburgers. This suggests that increased emulsification of the fat, i.e., the formation of smaller droplets by gelatin had some influence on the fat-holding ability of hamburgers only in this type of test.

Conclusions

The fat losses measured on frying for all types of hamburger patty were significantly less than those determined by the net test. The suggested cause of the lower fat losses measured by the frying method was the restricted migration of the fat through the dense protein matrix, while the net test included the migrable fat with the aid of centrifugation.

The fat in the hamburgers prepared with adipose tissue existed largely in the form of fat cells. These hamburgers had a better fat-holding ability on frying than hamburgers with rendered fat, the fat in the latter case existing in large fat pools and to a minor extent in small droplets. Such a difference in fat-holding can be explained by the fact that the collagenous network around the fat cells in the former type of hamburger contributes to the fat-holding. Using the net test, the difference is less, with regard to fat-holding, between the fat in the form of fat cells and fat pools.

The addition of an increased amount of connective tissue to hamburgers resulted in a more dense protein network, which gave the best fat-holding properties on frying. The dense protein network is believed to restrict the migration of the fat out of the product, thus controlling fat separation. The addition of large amounts of connective tissue did not improve the fat-holding properties, when measured by the net test. This indicates that the net test does not differentiate the fat that was trapped in the protein matrix from that migrated to the outside, that was measured by the frying test.

The addition of gelatin to the hamburgers, prepared with rendered fat, gave rise to smaller droplets, but the fat-holding on frying was not improved. The fat-holding properties, as determined by the net test, are in this case at their best, compared to the other systems evaluated. This suggests that the increased emulsification of fat with added gelatin can be partially responsible for increased fat stability in type E, compared to types A-D, when the influence of the transport of the fat on fat-holding is eliminated (i.e., the net test).
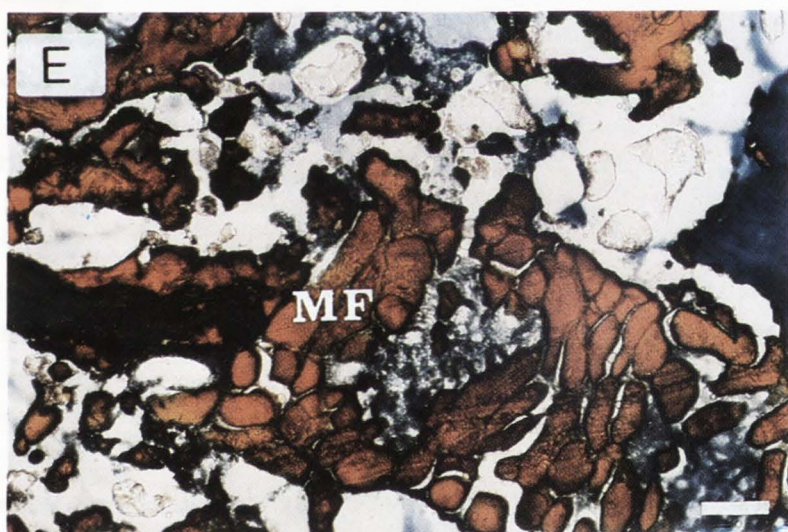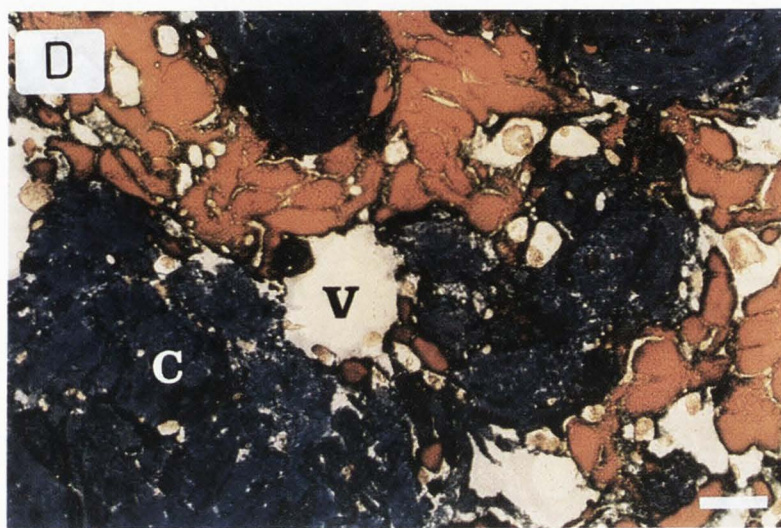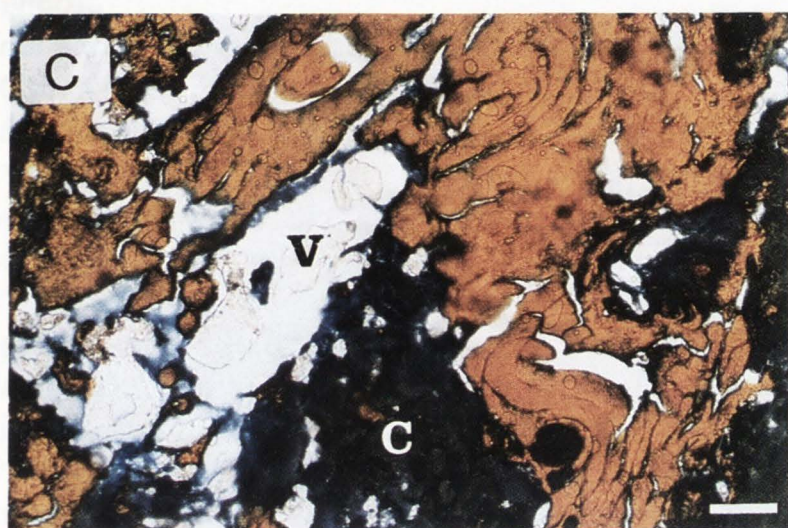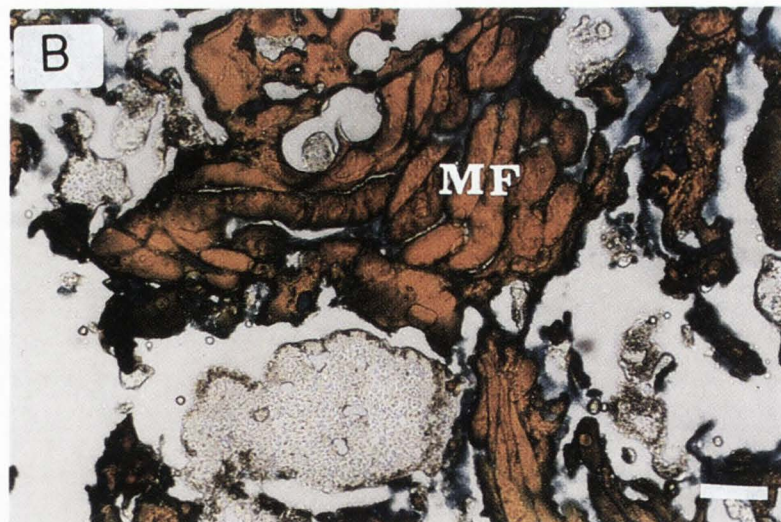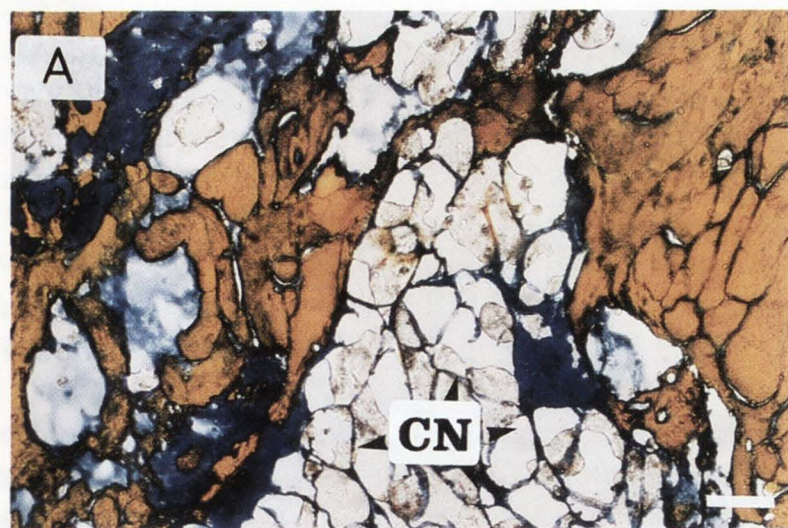
Figure 4 (color plate on facing page). Transverse sections of fried hamburger patties with A) adipose tissue, B) rendered fat, C) rendered fat + separated connective tissue, D) rendered fat + three times the amount of separated connective tissue and E) rendered fat + gelatin. Staining was performed with aniline blue and orange G. Bar = 100 μm; CN = connective tissue network; V = void; C = connective tissue; MF = muscle fiber.

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Acknowledgement

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Discussion with Reviewers

C.M. Lee: I do not see how emulsification occurs without extensive blending or shearing? Further does the micrograph show any interfacial film?

E. Puolanne: Is the statement in the text "This suggests that the emulsification of the fat has been facilitated by the addition of gelatin..." justified? If so, how do you define the term "emulsion"?

Authors: Emulsification takes place, when a protein goes to an interface and covers it. As gelatin was added to the hamburger it must exist in the water phase in the hamburgers. As the fat droplet size in E is less than in A (see Figures 2 and 3) nude fat/water interfaces are formed, due to comminution, and this is energetically unfavorable. Therefore gelatin, which is surface active,

goes to the interface and lowers the interfacial energy, which is the emulsification process. Therefore, fat in the form of fat pools is also emulsified, although not so efficiently as the fat in the form of small droplets.

F.W. Comer: From my experience, getting reproducible data from fat stability tests with coarsely ground meat products, such as hamburger patties is difficult because, presumably, these are not very homogeneous food systems. With reference to Figures 1 and 2, the highest correlations in Figure 1 (A, E) correspond to the most homogenous fat distribution shown in Figure 2. However, I am mostly interested in the similarities in Figures 1A and 1B as contrasted with the (expected) differences in Figures 2A and 2B. But for two values in each of the Figures for 1B, the data would be very similar to the corresponding Figures in 1A. In Figures 3A and 4A the protein membranes around the fat cells are still visible. This seems to indicate that the connective tissue in adipose tissue has very little influence upon fat stability in patties. Please comment on the role of adipose tissue protein membranes in fat stability of sausages.

Authors: We have examined the difference between fat-holding in hamburgers and sausages (Tornberg *et al.* 1989). The average droplet size of the fat was found to be 115 μm in hamburgers and 46 μm in sausages. Micrographs of the fat distribution in sausages and the size of the droplets indicated that most of the fat was squeezed out of the fat cells and existed in small fat pools. Therefore, the adipose tissue protein membrane probably is not located around the fat. The main fat-holding mechanism in sausages was found to be the mechanical entrapment in the protein matrix. The denser the network, the better the fat-holding ability. The fat content of the sausage was not the crucial factor regarding the fat-holding, as in the case of hamburgers. The protein from the adipose tissue might be a part of the network, and in that respect plays a role for the fat-holding ability in sausages.

F.W. Comer: The results from the "net test" indicate that gelatin improves fat stability (Figure 2E), and you have suggested an emulsification mechanism. To verify this, have you tried preparing a fat/gelatin/water pre-emulsion to determine whether further improvements in stability can be obtained? This procedure is used in cooked sausage, e.g., wieners, with sodium caseinate as the protein source, and I am interested whether it works in fresh sausage products.

Authors: We have not tried to prepare a pre-emulsion, but in Jones (1977) gelatin has been shown to be a good emulsifier.

F.W. Comer: The procedure that was used to isolate connective tissue from adipose tissue would be expected to result in some type of protein denaturation. Two hours heat treatment at 50°C and extensive chloroform washings might be expected to have some effect upon tertiary protein structure. Did you carry out more extensive heat treatment of the connective tissue to produce more gelatin as may be evidenced by a differential scanning calorimetry (DSC) thermogram?

Authors: The DSC thermograms (not shown here) of the adipose tissue as well as the micrographs revealed that the same contraction of the connective tissue did not occur until the temperature had reached 63 °C. This was the reason for the temperature of 50 °C to be selected for melting out part of the fat. The temperature (50 °C) might have influenced the non-collagen part of the connective tissue protein, but we were mainly interested in the collagen part of the connective tissue. We have further carried out heat treatment of the connective tissue (up to one hour) and did obtain an increased area of the suggested gelatin peak. Furthermore, heat treatment of the connective tissue (up to 8 hours) has been carried out and the formation of gelatin by measuring the amount of collagen in the water phase after the centrifugation was determined. The amount of collagen increased continuously (unpublished results) with time of the heat treatment. We hope to separately publish these results.

F.W. Comer: Part of the reason why fried patties are more stable than the "net test" results may be that lower internal temperatures are reached in the former. Did you determine the internal temperature? Were the patties still pink in the center which may result if fried from a frozen state?

Authors: The hamburger patties were fried from a frozen state. They were fried for 3.5 minutes on each side which resulted in a center temperature of about 70 °C (as given in the text). The patties were therefore not pink after being fried. The center temperature in the "net test" reached 77 °C after about 15 minutes, where the internal temperature was higher than in the fried hamburger. The temperature gradient was, however, larger when fried. Of course, the difference in center temperature and temperature gradient between the two types of tests can contribute to the differing fat losses obtained. But that is not the crucial thing, because two types were chosen, where in one case, both the instability of the fat *per se* and the transport of the fat out of the product comes into play in fat losses, whereas in the second type of test, the instability of the fat is the major factor controlling fat release. The way these two methods arrive at this situation is of less importance.

F.W. Comer: The stability results in Figure 1 can largely be explained by stating that the meat system can hold about 10-13% fat. To hold more fat requires the addition of ingredients or processing procedures which will increase either fat absorption or emulsification. In commercial practice, cereal, especially baked cereal, and protein ingredients are added for this purpose. From your results, connective tissue and gelatin are not particularly effective ingredients. Have you carried out experiments with other (non-meat) binder ingredients to determine their effects upon fat stability of hamburger patties?

Authors: We have so far worked mainly with the meat proteins in this area (hamburger patties). We have performed, though, some experiments with globin and fibers of beet as ingredients in hamburger patties and found that they mainly influenced the water-binding ability and did not improve the fat-holding properties. Our results so far suggest that the fat-holding of a coarse meat product, such as a hamburger, is mainly dependent upon how easily the fat can be transported out of the product on heating, and is much less dependent on the addition of different types of protein to the recipe.

E. Puolanne: You did use two different methods, the results of which you present parallel throughout the text, namely the behavior of different constituents of beef in hamburgers (practice-oriented) and the behavior of them in two different types of tests (theory-oriented). What is, according to you, the main result of your study?

Authors: The main observation to stress is that, due to the approach of using two type of cooking tests, we could, semi-quantitatively differentiate between the contribution of the protein matrix and fat instability to cooking fat losses. Together with the structural evaluation of the hamburgers this gave the possibility to speculate more on the mechanism of fat-holding in hamburgers, which is seldom seen in literature. Moreover, the results points out the danger in drawing general conclusions on fat-holding in hamburgers using only one type of test, as different type of tests reflects separate mechanisms in fat-holding.

E. Puolanne: How would you expect that pork fat would behave in similar tests, or do you think that all meat fat behave similarly at these cooking temperatures?

Authors: The difference in mechanism of fat-holding between the two type of methods tested will probably persist, while the fat loss values will differ. Probably lower fat losses will be obtained with pork fat compared to beef fat.

E. Puolanne: By frying, the relative fat loss showed a very strong increase as the fat content was increased. One hypothesis could be that the relative loss would have been constant in the rather narrow range of fat content given. A small increase seems logical, but I would like to know, how do you explain the sharp increase in fat loss. Could it have been so that in higher fat additions the amount of added water was too low to create a good binding for the mixture?

Authors: When the fat content of the hamburger is raised the water content is lowered, as the protein content is kept constant. This gives rise to a lower water/protein ratio in the hamburger, which is beneficial for the water-holding properties. For sausages, water losses and fat losses correlate well, whereas, this is not the case for hamburgers. In the Tornberg *et al.* (1989) paper, these phenomena have been thoroughly discussed ending up in the conclusion that in hamburgers fat separation is mainly controlled by the probability of encounter between droplets, which steeply rises as a function of fat content.

E. Puolanne: I understood from the Methods that the ingredients were only ground by the preparation of the hamburgers. What would have happened, if you had made a preemulsification of the fat tissues in the cutter before preparing the hamburgers? Did you think to compare chopping and grinding in the comminution during the preparation of the batter?

Did you vary the pressure in forming the patties? May it have had a different effect on different variables?

If you were to use preemulsification with e.g., soy protein or milk protein, what would have been the influence?

Authors: These are all excellent suggestiond for future work. At present, these questions cannot be answered until more experiments are performed.