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A NEW REFRACTOMETRIC METHOD OF INVESTIGATION FOR THE DISTINCTION BETWEEN FRESH AND FROZEN

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ABSTRACT:

A new method of differentiation between fresh and frozen meat is introduced, based on the measure of an indicator called "residual solid content of the blood". This freshly generated parameter derives from the index of refraction of the re-solubilized fraction in distilled water of the blood extracted from the edible that has interacted with the polypropylene of the Eppendorf Safe-Lock Tubes™, and it is measured through a simple hand-held refractometer. Aim of this research is to provide a simple, reproducible and inexpensive procedure of quality control investigation, especially designed for the application against the frauds in the tertiary sector.

KEY WORD: *Refractometry, Residual solid content of the blood, Quality Control, Frauds, Meat, Coagulation.*

INTRODUCTION:

The freezing is a method of preserving useful to increase the shelf life of meat and of food products in general. On the other hand, during thawing, the aliment undergoes the denaturation of proteins, the release of cellular content and exudation (Rahman, 2007). All these factors provoke a decrease in the organoleptic qualities of the frozen meat compared to the fresh one causing a reduction in the selling price and the possibility of fraud by dishonest traders, which are favored by the fact that the visible features (e.g. color, texture of the muscles and tissues) are similar. For another, the "Directive 2000/13/EC of the European parliament and of the council" of 20 March 2000, on the "approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs" says that "the name under which

the product is sold shall include or be accompanied by particulars as to the physical condition of the foodstuff or the specific treatment which it has undergone (e.g. powdered, freeze-dried, deep-frozen, concentrated, smoked) in all cases where omission of such information could create confusion in the mind of the purchaser". Therefore, to comply with this regulation, results very important the knowledge of the frozen\fresh status of the meat. For both these reasons, there is the need of a system designed for this distinction.

Refractometry is the method of measuring the refractive index of the substances in order to assess their composition or purity. The index of refraction of a substance is a dimensionless number that describes how light (or other radiations) propagates through that medium. The refractive index is expressed as $n = c/v$ where c is the speed of light in vacuum and v is the speed of light in the substance. A refractometer measures the different reaction to light depending upon the amount of solid that is available in the liquid sample. In other terms, in the Abbe based instrument used for this research, a drop of the sample solution is placed on a prism; the result is observed through an eyepiece. The critical angle (the angle beyond which light is totally reflected back into the sample) is a function of the refractive index and the operator detects this parameter by noting where a dark boundary falls on an engraved scale. The instrument contains a thermometer which can be used to correct to 20°C in situations where measurement cannot be made at exactly that temperature. The refractive index is expressed using the "Brix scale". The Brix degree (symbol °Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight (% w/w). If the solution contains dissolved solids other than pure sucrose, then the °Bx approximates the total dissolved solid content (Verlag Albert Bartens KG, 2010). In this research is analyzed an aqueous solution of blood components. This extract contains cells (such as erythrocytes, leukocytes and platelets) and plasma that is composed by water and organic substances such as carbohydrates (glucose), lipids (cholesterol, triglycerides, phospholipids, lecithin, fats), proteins (globulins, albumin, fibrinogen), glycoproteins, hormones (gonadotropins, erythropoietin, thrombopoietin), amino acids, vitamins and minerals in ionic form. Therefore, the °Bx value derives from the amount of all these solids in the solvent. Freezing is a physical reaction that involves the lowering of the temperature of the nourishment below 0° C, resulting in the gradual conversion of water, present in the meat, into ice. This conversion provokes an increase of the concentration of dissolved substances and a decrease of the water activity of the product. The crystallization process begins with a nucleus derived from a cluster of water molecules (formed when the temperature is reduced below 0° C). This core must be of a certain size to provide an adequate site for the crystal to begin to grow. If physical conditions are conducive to the presence of numerous seeds for crystallization, then a large number of small ice crystals will form. However, if only a few nucleus are initially available, then a few ice crystals will form and each will grow to a large size. Specifically, is the rate of freezing that determines their position and size (Grujic *et al.*, 1993). Fast freezing is conducive to

the formation of small ice crystals which are located intracellular and that cause few physical damage to meat components but that have a great effect on the blood cells. Slow freezing process favors large, extracellular, ice crystals to form, which results in disruption of muscle cells and, again, in a sensible effect on the blood cells. Several studies (Grujic *et al.*, 1993; Varnam *et al.*, 1995) show that frozen blood (derived from fast freezing) contains normal plasma levels of stable coagulation factors, albumin and immunoglobulins but a diminution of the quantities of other labile coagulation factors and naturally occurring inhibitors. Slow freezing, instead, causes a significant protein denaturation. On these basis is conceivable a diminution of the solid content of the blood in both the types of frozen meat if compared with that of the fresh meat. The fraud in the tertiary sector is more diffused for meat subjected to slow freezing. This research paper, thus, is focused on this typology of preservation method. Refractometry is widely used in the determination of the "blood total solid concentration" (Naylor *et al.*, 1977; Andreasen *et al.*, 1989) . It is the gold standard for the diagnosis of the "total protein" according to the "Merck veterinary manual for veterinary professionals" (Cynthia M. Kahn *et al.*, 2012). Despite of this, to distinguish fresh and frozen meat, other methods are used such as the measuring of electric resistance, enzymatic essays on α-glucosidase, β-N-acetylglucosaminidase and HADH, NMR spectroscopy on the NADH (Fernandez M *et al.*, 1999; Yuan CS *et al.*, 1988). On the other hand, refractometry is used in the determination of the hematocrit (Yoshioka K, 1983). The refractometric method for the determination of total protein in blood contemplates the analysis of the serum before and after protein is removed by coagulation (Wolf et al., 1962). This approach cannot be used to distinguish fresh and frozen meat due to the fact that the differences in the solid concentration are provoked not only by the proteins but also by the blood cells that are broken by ice crystal. Therefore, in blood extracted from fresh meat, the cells form the clot as they are, differently from the blood derived from frozen meat in which the destroyed corpuscles have different solubility. In this research is, thus, left to coagulate the blood. A direct measure of the index of refraction of this extract is not reproducible due to the high concentration of the solids. To counteract this factor is exploited the interaction of the particles of the clot with the walls of polypropylene of the Eppendorf Safe-Lock Tubes™ taking in consideration that at equal contact surface, the interaction will be directly proportional to the concentration of the solid in the sample.

MATERIALS AND METHODS:

Fresh adult bovine and swine meat are collected directly from a local butchery within one day of slaughter (they are stored in cold room at a variable temperature of 5°C-10°C). For the measures on thawed meat, aliquots of the same samples are previously frozen in "Whirpool freezer ARC 4020 IX". A "stainless steel press" is used to extract the blood from the meat (a normal "kitchen press" can be used for this purpose). The coagulation and the following solubilization of the solid particles encrusted on the polypropylene is

made in Eppendorf Safe-Lock Tubes™ 2ml. A RSGN-32ATC hand-held refractometer is used for the analysis.

Technical procedure

1. Blood from the flesh is extracted availing of the press. The obtained liquid is collected in Safe-Lock Tubes™ in portions of 1 ml (or 0.5 ml) for each tube.
2. The tube is left to coagulate for a period of 24h\72h.
3. The tube is emptied of all its content in order to separate the fraction of the clot encrusted on the polypropylene (this represents the indicator "residual solid content of the blood").
4. The "residual solid content of the blood" is solubilized in 0.5 ml of distilled water.
5. This solution is analyzed with a "Abbe refractometer". The model used in this research is "RSGN-32ATC hand-held refractometer". Experimentally, this instrument is firstly calibrated using distilled water. (See table 1 for technical details).
6. The data is statistically analyzed to obtain a "reference table".

RESULTS AND DISCUSSION:

Separate Brix measures of the "residual solid content of the blood" are taken for bovine and pork meat, fresh and frozen, within 24 hours or 72 hours from the start of the coagulation and according to the different kinds of the slaughtered meat. Each parameter is subjected to several measures, on different samples and in different days, so as to consider the biological variability. Tables from 2 to 9 show the data obtained.

The statistical analysis for the validation of data is aimed to explain different factors. Firstly, understand if the values of Brix absorption for the "residual solid content of the blood" derived from 0.5 ml and 1 ml of extract correlate, in order to make independent the result from the volume of the clot. The relationship is evaluated through the function "scatter chart" in Excel 2007. On the Y axis there is the "Average of the Brix absorption at 20°C" (the mean is obtained using together the measures of coagulation within 24h and 72h), on the X axis the volume of the clot (0.5 or 1 ml). Figures 1 to 4 and Table 10 show the derived graphs and formulas.

As noticeable from the charts and from the equations of correlations, in all the cases there is a positive linear relationship. Is, then, possible to affirm that the "residual solid content of the blood" is independent from the volume of coagulated blood. A second study on the data is intended to understand if there is a statistically significant difference in the quantity of "residual solid content of the blood" in the blood's extracts left to coagulate 24h or 72h. An analysis using the paired t-test is conducted. The paired t test compares the means of two matched groups, assuming that the distribution of the before-after differences follows a Gaussian distribution. Is essential to use the "paired t test" due to the fact that the subjects of the analysis are the same.

The formula is:

$$t = \frac{X_i - X_j}{\sqrt{\frac{s_i^2 (n_i - 1) + s_j^2 (n_j - 1)}{n_i + n_j - 2} \left(\frac{1}{n_i} + \frac{1}{n_j}\right)}}$$

where: X_i and X_j are the average of the variable of interest in the two analyzed groups, s_i^2 and s_j^2 are the variance of the two samples on the variable of interest and n_i and n_j are the number of elements for each of the two groups. The result is interpreted using *p*-values. Calculation are made using QuickCalcs of "GraphPad software" (http://www.graphpad.com/quickcalcs/ttest1/).

As shown in table 11, considering 0.05 as significance level, the various periods of coagulation produce a statistically significant variation in the "residual solid content of the blood" in three out of sixteen cases. On the other hand, considering 0.01 as significance level, no result is significant. Is then possible affirm that the Brix measure is independent from the period of formation of the clot.

Finally, the same statistical analysis is used to evaluate the significance of the difference in the Brix absorption between fresh and frozen meat.

Overall, following the procedure described in the paragraph "materials and methods" regardless of whether the analysis is carried out on 1 ml or 0.5 ml of blood, and being careful to leave it precipitate up to a maximum of 72 hours, the value of the "residual solid content of the blood" gives information on the kind slaughtered meat. Table 13, then, can be used as a reference to take information on the sample according to its "Brix absorption" favored also by the fact that the differences in the "residual solid content of the blood" of the samples have a range such as to ensure the reproducibility of the measurements.

CONCLUSION:

The characteristics of the analysis of the "residual solid content of the blood" are the simplicity of the instrumentation used, the short time required for the essays and affordability. Further, is not requested technical expertise. These features make this method really useful in the quality control investigations for both the operators of the tertiary industry, with the aim of comply with the food-related lawful directives, and for the qualified staff of the supervisory bodies. Additional investigations on other animal species intended for slaughter can extend the "reference table" derived from this research paper, making this method even more effective.

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REFRENCES:

Andreasen CB, Latimer KS, Kircher IM, Brown J. (1989): Determination of chicken and turkey plasma and serum protein concentrations by refractometry and the biuret method. *Avian Diseases*, 33(1), 93-106.

A.V. Wolf, John B Fuller, E.J. Goldman, T.D. Mahony. (1962): New Refractometric Methods for the Determination of Total Proteins in Serum and in Urine. *Clinical Chemistry*, 8(2), 158-165.

Cynthia M. Kahn, Scott line. (2010): The Merck veterinary manual (10th edition). New Jersey: MERCK MANUALS, 2593 p. ISBN 978-0-91191-093-3.

Fernandez M., Mano S., Garcia de Fernando GD, Ordonez ja, Hoz L. (1999): Use of β-hydroxyacyl- CoA-dehydrogenase (HADH) activity to differentiate frozen from unfrozen fish and shellfish. *European Food Research Technology*, 209, 205–208.

Grujic R., L.Petrovic, B. Pikula and L.Amidzic. (1993): Definition of the optimum freezing rate. I. Investigation of structure and ultra structure of beef M. longissimus dorsi frozen at different freezing rates. *Meat Science*, 33, 301-318.

M. Shafiur Rahman. (2007): Handbook of Food Preservation, Second Edition. Boca Raton: CRC PRESS, 1088p. ISBN 978-1-57444-606-7.

Naylor JM, Kronfeld DS. (1977): Refractometry as a measure of the immunoglobulin status of the newborn dairy calf: comparison with the zinc sulfate turbidity test and single radial immunodiffusion. *Am J Vet Res*, 38, 1331–1334.

Varnam A., Sutherland Jane p. (1995): Meat and Meat products: Technology, Chemistry and Microbiology. London: CHAPMAN AND HALL, 430p. ISBN 978-0-41249-560-1.

Verlag Albert Bartens KG. (2010): Icumsa Methods Book. Berlin: ICUMSA, 341p. ISBN: 978-3-87040-553-3.

Yoshioka K. (1983): Differentiation of freeze-thawed fish from fresh fish by the determination of hematocrit value. *Bull Jpn Soc Sci Fish*, 54, 149-151.

Yuan CS, Yoshioka K, Ueno R. (1988): Differentiation of frozen-thawed fish from unfrozen fish by determination of neutral β-N-acetylglucosaminidase activity in the blood. *Bull Jpn Soc Sci Fish*, 54, 2143-2148.

Table 1: Operation instructions for refractometer

OPERATION INSTRUCTIONS:
Calibration: Open the "daylight plate" and place 2-3 drops of distilled water on the "prism". Close the "daylight plate" so that the water spread across the entire surface of the "prism". Hold the "daylight plate" in the direction of a light source and look into the "eyepiece". Will be possible to see a circular field with graduation scale. The upper portion of the field should be blue, while the lower portion should be white. To calibrate, turn the "calibration screw" (or correct screw) until the boundary between the upper blue field and the lower white field meets exactly on the zero scale. Under the "calibration screw" there is the "calibration lock". To tighten the lock, turn the "calibration lock" clockwise.
Measure: Place 2-3 drops of the specimen of liquids instead of the distilled water on the "daylight plate" and look into the "eyepiece" in the direction of a light source. Will be possible to take the reading where the boundary line of blue and white cross the graduate scale. The scale will provide a direct reading of the concentration.

Table 2: Brix absorption of 1 ml of fresh bovine meat

	Brix values		**Average of the brix values**		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Chuck steak	2.0 -1.8-1.8-2.0 (±0.2)	2.0-2.0-2.2-2.0 (±0.2)	1.925	2.075	2
	2.0-2.0-1.8-2.0 (±0.2)	2.2-2.0-2.0-2.2 (±0.2)			
Shank (around the knee)	1.4-1.6-1.8-1.8 (±0.2)	1.8-2.0-2.2-1.8 (±0.2)	1.65	1.925	1.8
	1.6-1.6-1.8-1.6 (±0.2)	2.0-1.8-2.0-1.8 (±0.2)			

Table 3: Brix absorption of 0.5 ml of fresh bovine meat

	Brix values		**Average of the brix values**		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Chuck steak	0.6 -0.8-0.8-1.0 (±0.2)	0.8-1.0-1.2-1.0 (±0.2)	0.9	1.05	0.98
	1.0-0.8-1.2-1.0 (±0.2)	1.2-1.0-1.0-1.2 (±0.2)			
Shank (around the knee)	0.6-0.8-0.8-0.8 (±0.2)	0.8-1.0-1.2-0.8 (±0.2)	0.85	0.925	0.89
	1.0-1.0-0.8-1.0 (±0.2)	1.0-0.8-1.0-0.8 (±0.2)			

Table 4: Brix absorption of 1 ml of frozen bovine meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Chuck steak	1.0 -1.0-1.2-1.0 (±0.2)	1.2-1.4-1.2-1.2 (±0.2)	0.975	1.175	1.075
	0.8-0.8-1.0-1.0 (±0.2)	1.2-1.0-1.0-1.2 (±0.2)			
Shank (around the knee)	1.0-1.2-1.2-0.8 (±0.2)	1.4-1.0-1.2-1.2 (±0.2)	1.025	1.125	1.075
	1.0-1.0-0.8-1.2 (±0.2)	1.0-1.0-1.0-1.2 (±0.2)			

Table 5: Brix absorption of 0.5 ml of frozen bovine meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Chuck steak	0.6-0.4-0.6-0.6 (±0.2)	0.8-0.4-0.6-0.6 (±0.2)	0.525	0.65	0.59
	0.4-0.6-0.6-0.4 (±0.2)	0.6-0.6-0.8-0.8 (±0.2)			
Shank (around the knee)	0.4-0.6-0.6-0.8 (±0.2)	0.6-0.6-0.8-0.8 (±0.2)	0.625	0.7	0.66
	0.6-0.6-0.6-0.8 (±0.2)	0.8-0.8-0.6-0.6 (±0.2)			

Table 6: Brix absorption of 1 ml of fresh pork meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Shoulder steak	3.0-3.0-2.8-3.2 (±0.2)	3.2-3.4-3.2-3.0 (±0.2)	2.975	3.15	3.06
	2.8-3.0-3.0-3.0 (±0.2)	3.2-3.0-3.0-3.2 (±0.2)			
Loin joint	3.0-3.2-3.2-2.8 (±0.2)	3.4-3.0-3.2-3.2 (±0.2)	2.975	3.125	3.05
	2.8-3.0-2.8-3.0 (±0.2)	3.0-3.0-3.0-3.2 (±0.2)			

Table 7: Brix absorption of 0.5 ml of fresh pork meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Shoulder steak	1.2-1.6-1.6-1.4 (±0.2)	1.4-1.6-1.6-1.4 (±0.2)	1.475	1.525	1.5
	1.4-1.6-1.6-1.4 (±0.2)	1.6-1.4-1.6-1.6 (±0.2)			
Loin joint	1.4-1.6-1.6-1.4 (±0.2)	1.4-1.6-1.6-1.6 (±0.2)	1.475	1.5	1.49
	1.2-1.6-1.4-1.6 (±0.2)	1.4-1.4-1.4-1.6 (±0.2)			

Table 8: Brix absorption of 1 ml of frozen pork meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Shoulder steak	1.8-1.6-2.0-2.0 (±0.2)	2.0-1.8-2.2-2.0 (±0.2)	1.925	2	1.96
	1.8-2.0-2.2-2.0 (±0.2)	2.2-2.0-1.8-2.0 (±0.2)			
Loin joint	1.8-2.0-2.0-2.2 (±0.2)	2.4-2.0-2.0-2.2 (±0.2)	1.975	2.15	2.06
	1.8-2.0-2.0-2.0 (±0.2)	2.4-2.2-2.0-2.0 (±0.2)			

Table 9: Brix absorption of 0.5 ml of frozen pork meat

	Brix values		Average of the brix values		
	measured within 24h of coagulation	measured within 72h of coagulation	24h	72h	tot
Shoulder steak	1.0-1.0-1.2-1.2 (±0.2)	1.0-1.2-1.2-1.0 (±0.2)	1.025	1.1	1.06
	1.0-1.0-0.8-1.0 (±0.2)	1.2-1.2-1.0-1.0 (±0.2)			
Loin joint	1.0-1.0-1.0-1.2 (±0.2)	1.4-1.0-1.0-1.2 (±0.2)	1.05	1.15	1.1
	1.2-1.0-1.0-1.0 (±0.2)	1.4-1.2-1.0-1.0 (±0.2)			

Table 10: Equations of correlation "Brix absorption \ volume of the clots

Sample	Equations of correlation
Fresh bovine "Chuck steak"	Y = 0.49*X + 0.02
Fresh bovine "Shank"	Y = 0.55*X + 0.01
Frozen bovine "Chuck steak"	Y = 1.03*X - 0.11
Frozen bovine "Shank"	Y = 1.20*X - 0.29
Fresh pork "Shoulder steak"	Y = 0.32*X + 0.02
Fresh pork "Loin joint"	Y = 0.32*X + 0.02
Frozen pork "Shoulder steak"	Y = 0.56*X - 0.09
Frozen pork "Loin joint"	Y = 0.52*X - 0.07

Table 11: Statistical analysis of the difference regarding the period of coagulation

Sample	Volume	Two-tailed "p-value"	Significance level	
			0.05	0.01
Fresh bovine "chuck steak"	1 ml	0.1723	N	N
Fresh bovine "shank"	1 ml	0.0103	Y	N
Fresh bovine "chuck steak"	0.5 ml	0.0941	N	N
Fresh bovine "shank"	0.5 ml	0.3559	N	N
Frozen bovine "chuck steak"	1 ml	0.0453	Y	N
Frozen bovine "shank"	1 ml	0.6891	N	N
Frozen bovine "chuck steak"	0.5 ml	0.0941	N	N
Frozen bovine "shank"	0.5 ml	0.1996	N	N
Fresh pork "shoulder steak"	1 ml	0.0941	N	N
Fresh pork "loin joint"	1 ml	0.4072	N	N
Fresh pork "shoulder steak"	0.5 ml	0.3559	N	N
Fresh pork "loin joint"	0.5 ml	0.3506	N	N
Frozen pork "shoulder steak"	1 ml	0.4423	N	N
Frozen pork "loin joint"	1 ml	0.0639	N	N
Frozen pork "shoulder steak"	0.5 ml	0.1970	N	N
Frozen pork "loin joint"	0.5 ml	0.0331	Y	N

Legend: Y - statistically significant difference, N - not statistically significant difference

Table 12: Statistical analysis of the differences according to the physical state (fresh\frozen) of the samples

Sample	Volume	Period of coagulation	Two-tailed "p-value"	Difference (at 0.05 significance level)
Bovine "chuck steak"	1 ml	24 h	less than 0.0001	extremely statistically significant
Bovine shank"	1 ml	24 h	less than 0.0001	extremely statistically significant
Bovine "chuck steak"	0.5 ml	24 h	0.0022	very statistically significant
Bovine shank"	0.5 ml	24 h	less than 0.0001	extremely statistically significant
Bovine "chuck steak"	1 ml	72 h	less than 0.0001	extremely statistically significant
Bovine shank"	1 ml	72 h	less than 0.0001	extremely statistically significant
Bovine "chuck steak"	0.5 ml	72 h	less than 0.0001	extremely statistically significant
Bovine shank"	0.5 ml	72 h	0.0379	statistically significant
Pork "shoulder steak"	1 ml	24 h	less than 0.0001	extremely statistically significant
Pork "loin joint"	1 ml	24 h	less than 0.0001	extremely statistically significant
Pork "shoulder steak"	0.5 ml	24 h	0.0002	extremely statistically significant
Pork "loin joint"	0.5 ml	24 h	0.0011	very statistically significant
Pork "shoulder steak"	1 ml	72 h	less than 0.0001	extremely statistically significant
Pork "loin joint"	1 ml	72 h	less than 0.0001	extremely statistically significant
Pork "shoulder steak"	0.5 ml	72 h	less than 0.0001	extremely statistically significant
Pork "loin joint"	0.5 ml	72 h	0.0092	very statistically significant

Table 13 Reference table

Sample	“residual solid content of the blood” in 100 grams of solution	Brix absorption of the acqueous solution derived from 1 ml of coagulated blood	Brix absorption of the acqueous solution derived from 0.5 ml of coagulated blood
Fresh Bovine meat	~2 g	~2° Bx	~1° Bx
Frozen Bovine meat	~1 g	~1° Bx	~0.5° Bx
Fresh Pork meat	~3 g	~3° Bx	~1.5° Bx
Frozen Pork meat	~2 g	~2° Bx	~1° Bx

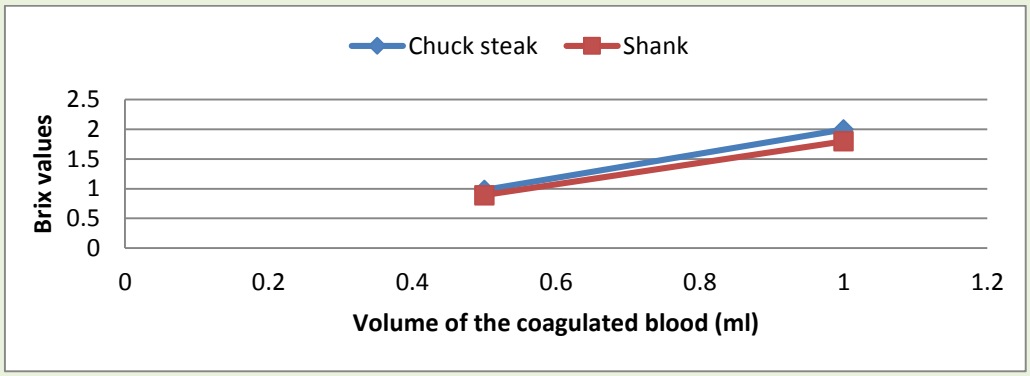

Figure 1: Correlation graphs "Brix absorption \ volume of the clots" for fresh bovine meat

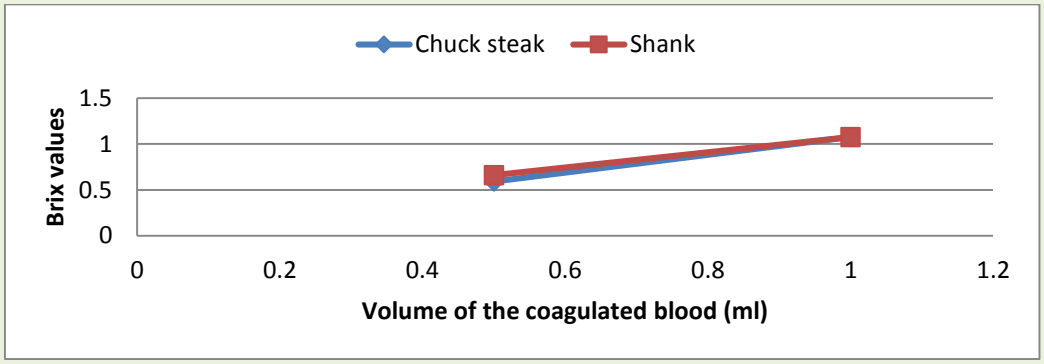

Figure 2: Correlation graphs "Brix absorption \ volume of the clots" for frozen bovine meat

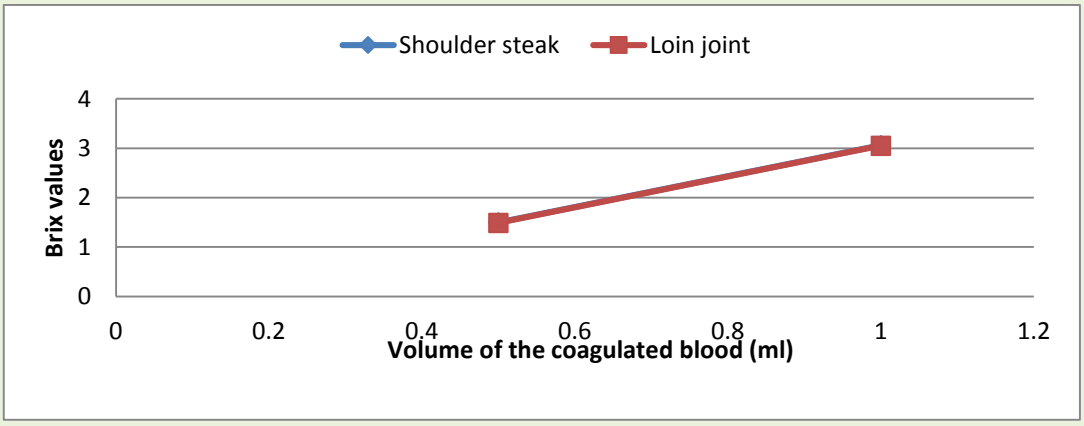

Figure 3: Correlation graphs "Brix absorption \ volume of the clots" for fresh pork meat

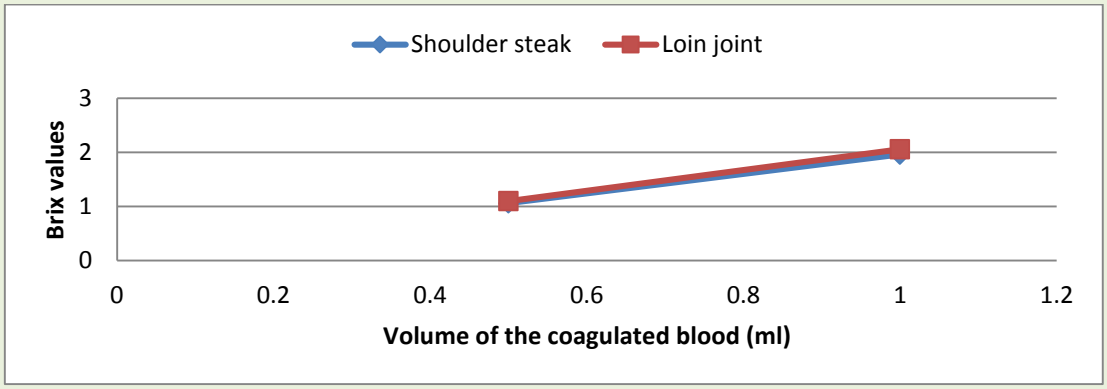

Figure 4: Correlation graphs "Brix absorption \ volume of the clots" for frozen pork meat