CONTRIBUTION OF THE MAJOR CONSTITUENTS TO THE TOTAL REFRACTION IN MILK

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Investigations on refraction in milk[1, 2], and the relation between refraction and freezing point of milk[3] has been reported in earlier papers. Refractive index of milk is made up of contributions from proteins and solutions of lactose, mineral salts and other minor constituents. Fat has already been shown to contribute nothing to the refractive index[1, 2] owing, evidently, to its gross particulate size and physical state of suspension in milk. The share of the other constituents has not been so far ascertained, since there was, up to now, no way of estimating the value of refractive index of the mammary secretion which include the colloidal proteins. The present method[1] therefore enables the apportionmment of the role of the principal constituents by successively eliminating the ingredients from the liquid.

Studies on the relationship between the refractive index and non-fatty solids of milk proved that the two are not related in an exactly linear fashion[1]. In other words, for a given value of refractive index the corresponding value of fat-free solids varies over a small range instead of being a constant value, and *vice versa*. The refractive index being due to the soluble (including colloidal) solids in milk, the lack of an exact linear relationship between the two could only be accounted for by the variations in the mutual proportions of the contributory constituents — chiefly proteins, milk sugar and soluble salts. Further, the fact that the linear relationship between the freezing point of milk and the refractive index of *milk serum*, is lacking between freezing point and refractive index of *milk*[3] proves that solids other than those that are in true solution in milk come into play in the refractive index of milk.

The nearest approach to this aspect of the problem was by BAIER and NEUMANN[4] (1907) who reported that refractive index of the calcium chloride serum could be used as a measure of the lactose content of the milk. Later, PANCHAND and AVERBACH[5] reported that the change in the refractometric readings of milk serum and gravimetric results of lactose in normal fresh and watered milk agree remarkably. But it will be observed that this provides no argument for concluding that the refraction in the serum is a direct and the sole measure of the lactose content of milk, for the other constituents of milk serum, like unprecipitated proteins, and mineral salts are all diluted in the same proportion as milk sugar.

SCHULZE[6] made an observation that the refraction of serum and lactose content undergo corresponding fluctuations in herd milks, and concluded that refraction gives sufficient indication of the lactose content.

In this paper, therefore, the results of experiments conducted not only with a view to fixing the share of each of the principal constituents in the total magnitude of refrac-

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tion, but also the results of experiments on the relationship between the refraction in milk or copper serum and the percentage of lactose in the milk, are presented.

EXPERIMENTAL PROCEDURE

In order to study the relationship between the lactose content, the refractive index of milk and of the copper serum of milk, samples of fresh cow and buffalo milk were first tested for density and refractive index as before[1]. Then the copper serum was prepared by filtering a well-shaken mixture of 4 parts of milk and 1 part of copper sulphate solution (72.5 g of copper sulphate crystals in 1 liter) refracting at 36 at 20° C in the immersion refractometer as prescribed by the A.O.A.C.[7] procedure. The serum refraction of samples was then tested in the Abbé refractometer applying the temperature correction worked out by ELSDON and STUBBS[8]. The fat content of the sample was estimated by the GERBER process. Table II gives the resulting data.

The individual role of the several constituents of milk in the total refractive index have to be studied, it will be admitted, by methods of separation of the constituents that do not interfere with the magnitude of refraction of the product in question. Thus, while removal of fat by centrifugation does not alter the refraction, extraction with ether does. Similarly, acid or heat coagulation of the proteins cannot be resorted to, as such treatment considerably alters the refraction. Methods, mostly physical, were therefore adopted for the successive elimination of the principal constituents.

Individual as well as bulk samples of cow milk were used for these studies. The total solids, fat content, milk sugar[9] and ash of the sample were first tested as usual by the standard methods. Then the refractive index of the milk was tested as described before.

In order to eliminate the casein without altering the refractive index of the resulting serum, a few trials were made to test the effect of added liquid rennet (HANSEN's, R.I. 20° C, 1.3690) on the refractive index of milk, by estimating the refraction immediately before and after addition of different proportions of rennet to skim milk. Finally, it was found that one drop of the rennet in 25 ml of milk caused no detectable difference in the value of the refractive index of milk.

One drop of HANSEN's liquid rennet was added, therefore, to 25 ml of the milk for curdling the casein. When the milk set, after about 30 minutes, it was filtered on a dry, grade 5 Whatman filter paper. The clear filtrate was tested for refractive index, by making use of just one drop of the filtrate.

The casein curd was repeatedly macerated with about 5 ml of distilled water and filtered through the same filter until the filtrate was quite clear and did not reduce FEHLING's. This indication of the absence of milk sugar in the final filtrate was taken to mean that the lactalbumin and globulin in the rennet serum[10, 11] had also been extracted from the curd along with the milk sugar.

The filtrate, collected in a Volumetric flask, was made up to 100 ml with distilled water and well shaken. The fat content of the extract was estimated by the GERBER process using a butyrometer calibrated for buttermilk. The total solids was determined as before by evaporating off the moisture, and the ash content by incineration in the usual fashion.

Finally, in order to eliminate the lactalbumin and globulin along with the casein, about 5 ml of milk was accurately weighed and subjected to ultrafiltration, using a cellophane filter, at 20 kg per cm² pressure. Just one drop of the ultrafiltrate was used for estimating its refraction with the Abbé refractometer. The precipitate on the filter was then repeatedly extracted with 2 ml of distilled water containing a drop of chloroform. The latter was added to check acidity development in the milk from bacterial action in the course of the filtration which ordinarily took from 2 to 4 hours. The filtration was stopped as soon as the final drops ceased to reduce Fehling's, assuming, in this instance, that all other minor crystalloidal constituents of milk were extracted into the filtrate along with lactose. The entire ultrafiltrate was then evaporated to dryness to determine the total solids in it, and finally ashed for ash content.

The ultrafiltration, separately, of milk as well as of its rennet serum (with one drop of rennet in 25 ml of milk) proved that the two filtrates were identically free from lactalbumin and globulin inasmuch as they had the same refractive index. This fact is clear from the following table.

TABLE I

REFRACTIVE INDEX OF ULTRAFILTRATE FROM MILK AND FROM RENNET-SERUM OF MILK

R.I. (40° C)		R.I. (40° C) Ultrafiltrate from	
Milk	Rennet-serum	Milk	Rennet-serum
1.3478	1.3421	1.3384	1.3383
1.3452	1.3421.	1.3381	1.3382

The anlytical data of the preceding experiments are given in Tables III, IV and V.

TABLE II

RELATIONSHIP BETWEEN REFRACTION IN MILK, COPPER SERUM AND LACTOSE OF MILK

Density (20° C)	Fat %	Lactose %	R.I. (40° C)	
			Milk	Copper serum
		Cow Milk		
1.0290	5.3	4.88	1.3464	1.3318
277	4.7	4.20	55	18
279	—	4.60	59	18
280	5.1	4.47	56	19
278	5.2	4.64	60	—
293	4.3	4.70	61	—
—	—	4.49	1.3463	—
1.0289	6.0	4.52	73	1.3318
		Buffalo Milk		
1.0269	6.7	5.30	1.3463	1.3319
273	6.1	4.70	76	18
302	—	4.70	81	15
242	6.4	4.08	62	19
264	6.9	4.80	62	—
260	·6.8	4.36	62	—
—	—	4.64	79	—

It is clear from Table II that the refractive index of the copper serum varies very little compared to that of milk or of the content of milk sugar. From the latter it follows that the refractive index of the copper serum cannot be taken as a measure of the lactose content of milk, which therefore fails to support the observation of SCHULZE (*loc. cit.*).

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It also follows that, the greater constancy of the refractive index of the serum having resulted from the elimination of the protein from milk, this constituent is responsible for the wider range of variation of the refractive index of *milk* (as fat does not contribute anything to the constant).

TABLE III

COMPOSITION AND CONSTANTS OF MILK, RENNET-SERUM AND ULTRA-FILTRATE OF COW MILK
(PERCENTAGES ARE EXPRESSED ON THE BASIS OF 100 g OF MILK)

	Milk	Rennet-serum	Ultra-filtrate
Sample I:			
Density (20° C)	1.0298	—	—
Total solids %	14.59	7.00	6.00
Fat %	5.50	0.4	0.0
Lactose %	4.8	4.8	4.8
Ash %	0.85	0.60	0.50
R.I. (20° C)	1.3495	1.3442	1.3414
Sample II:			
Density (20° C)	1.0292	—	—
Total Solids %	14.63	5.40	—
Fat %	5.80	0.15	0.0
Lactose %	4.76	4.76	4.76
Ash %	0.72	0.46	—
R.I. (20° C)	1.3498	1.3443	1.3408
Sample III:			
Density (20° C)	1.0287	—	—
Total Solids %	15.25	6.4	5.61
Fat %	6.6	0.27	0.0
Lactose %	4.63	4.63	4.63
Ash %	0.73	0.46	0.404
R.I. (20° C)	1.3495	1.3439	1.3416
Sample IV:			
Density (20° C)	1.0294	—	—
Total Solids %	14.28	9.19	5.57
Fat %	5.90	—	0.0
Lactose %	4.56	4.56	4.56
Ash %	0.75	0.43	—
R.I. (20° C)	1.3485	1.3436	1.3415
Sample V:			
Density (20° C)	1.0310	—	—
Total Solids %	15.02	6.42	5.62
Fat %	5.95	0.16	0.0
Lactose %	4.86	4.86	4.86
Ash %	0.71	0.46	0.403
R.I. (20° C)	1.3506	1.3446	1.3413

In Table III the content of casein and, lactalbumin + globulin, are deduced from the difference between the total solid content of milk, rennet-serum and ultra filtrate. The contribution of these fractions towards the refractive index are similarly deduced by the corresponding differences between the refractive indexes of the respective liquids. The refractive index of the ultrafiltrate is again resolved between that of lactose and of minor constituents of milk including dissolved inorganic salts, non-protein nitrogen, citric acid, vitamins, etc. The lactose content of the sample having been separately estimated, the refractive index of a solution (in this case, milk) containing that percen-

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tage of sugar is obtained from standard tables[12]. Therefore the excess of the value of the refractive index of the ultra-filtrate over that of the solution of lactose is contributed by the minor constituents. The following table thus gives the share of each of the gross fractions of the total solids of milk in raising the refractive index of water when they exist in the various physical states obtaining in fresh milk.

TABLE IV

CONTRIBUTION OF THE MAJOR CONSTITUENTS TO THE REFRACTIVE INDEX OF COW MILK

Constituent	% in milk	Contribution to R.I.
Sample I:		
Milk	—	1.3495 (20° C)
Water	—	1.3330 (20° C)
Total Solids	15.25 (S.N.F. 8.65)	0.0165
Fat	6.6	0.0
Casein	2.52	0.0056 } Proteins
Lactalbumin-globulin	0.52	0.0023 } 0.0079
Lactose	4.63	0.0064*
Minor	0.98	0.0022
Sample II:		
Milk	—	1.3485 (20° C)
Water	—	1.3330 (20° C)
Total Solids	14.28 (S.N.F. 0.00)	0.0155
Fat	5.90	0.00
Casein	2.28	0.0049 } Proteins
Lactalbumin-globulin	0.53	0.0021 } 0.0070
Lactose	4.56	0.0063*
Minor	1.01	0.0022
Sample III:		
Milk	—	1.3506 (20° C)
Water	—	1.0330 (20° C)
Total Solids	15.02 (S.N.F. 9.07)	0.0176
Fat	5.95	0.00
Casein	2.21	0.0060 } Proteins
Lactablumin-globulin	0.64	0.0033 } 0.0093
Lactose	4.86	0.0067*
Minor	0.76	0.0016
Sample IV:		
Milk	—	1.3495 (20° C)
Water	—	1.3330 (20° C)
Total Solids	14.39	0.0165
Fat	5.5	0.00
Casein	2.46	0.0053 } Proteins
Lactalbumin-globulin	0.60	0.0028 } 0.0081
Lactose	4.80	0.0066*
Minor	1.20	0.0018
Sample V:		
Milk	—	1.3498 (20° C)
Water	—	1.3330 (20° C)
Total Solids	14.63	0.0168
Fat	5.8	0.00
Casein	2.58	0.0055 } Proteins
Lactalbumin-globulin	—	0.0035 } 0.0090
Lactose	4.76	0.0065*
Minor	—	0.0013

* Value obtained from sugar table in BROWNE's *Handbook of Sugar Analysis.*

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DISCUSSION

It is clear from Table IV that the largest individual share of the refractive index is contributed by the proteins (casein + lactalbumin + globulin), next by lactose, and least by minor constituents including soluble salts. In proportion to their percentage in milk also, the proteins are the largest contributors, and the minor constituents rank next and lactose last in this regard. Among the proteins, it will be observed, the lactalbumin and globulin contribute, considering their proportion, very much more than casein to the refractive index of milk.

ROBERTSON[13] was the first to determine the refractive index of a solution of casein in 0.1 N NaOH. He gave a value of 0.00152 of refractive index for 1 g of casein in 100 ml of solution. With an improved technique and a purer sample of casein, BRERETON AND SHARP[14] obtained a higher value — 0.00181 per g in 100 ml. The present series of experiments consistently give a refraction of 0.0021 per g of casein in 100 ml of milk in all samples. This higher value over that of the earlier workers is perhaps to be attributed to (i) the greater dispersity of casein in milk then in a solution of sodium hydroxide and (ii) the difference between the chemical composition of the casein in milk (Calcium caseinate) and in sodium hydroxide.

Further, it is also clear from the table that the share of milk sugar in the refractive index is the most constant of all the ingredients of milk, and that the variability of the proteins is largely responsible for the wide changes in refractive index of milk from sample to sample.

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SUMMARY

The refractive index of milk being made up by contributions from several constituents, the share of each of the major constituents has been estimated by adopting methods of successive elimination of the fractions that do not interfere with the refraction in the resulting liquids.

Centrifugation, coagulation of casein with a minute quantity of rennet and ultra-filtration have been pressed into service for achieving this object. The difference between the refractive indices of the resulting liquids fixes the contribution of the constitutents so eliminated in the process.

In the falling order, the proteins, lactose and minor constitutents contribute to the total refraction in milk. (Fat has no share in this phenomenon). Of these, in proportion to their presence in milk, lactalbumin and globulin contribute the greatest share, more than that of casein.

A control experiment to verify SCHULZE's observation that the refractive index of milk serum is an indication of its sugar content has also been conducted.

Milk sera obtained with copper sulphate solution or rennet as precipitants show that no linear relationship exists between the refractive index of the serum and the lactose content of milk.

The data obtained in all the above experiments show that the wide variability of the refractive index of milk is due mainly to the variations in its protein content.

RÉSUMÉ

L'indice de réfraction du lait étant dû à différents constituants, la part de chacun d'eux dans ce phénomène a été déterminée par élimination successive de différentes fractions par des méthodes n'apportant aucune modification secondaire dans la réfraction des liquides résultants.

Ces méthodes sont essentiellement la centrifugation, la coagulation de la caséine par une quantité minime de présure, et l'ultrafiltration. La différence observée entre les indices de réfraction des liquides résultants montre quelle est l'importance des constituants éliminés.

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Par ordre décroissant, les protéines, le lactose et les constituants mineurs contribuent à la réfraction totale du lait (la graisse ne joue ici aucun rôle). Le rôle le plus important par rapport à leur quantité dans le lait est joué par la lactalbumine + globuline. Ce rôle est plus important que celui de la caséine.

Des expériences ont été faites, destinées à vérifier l'observation de SCHULZE d'après laquelle l'indice de réfraction du lait est une indication de sa teneur en sucre. Du sérum de lait obtenu après précipitation à l'aide d'une solution de sulfate de cuivre et de présure, montre qu'il n'existe aucune proportionnalité entre l'indice de réfraction du sérum et la teneur en lactose du lait.

Toutes les données obtenues au cours des expériences du présent travail montrent que la grande variabilité de l'indice de réfraction du lait est due essentiellement à des variations de sa teneur en protéines.

ZUSAMMENFASSUNG

Da der Brechungsindex von Milch aus Beiträgen mehrerer Bestandteile zusammengestellt ist, wurde der Anteil aller Hauptbestandteile dadurch bestimmt, dass Methoden angewandt wurden, um die Bestandteile der Reihe nach zu entfernen. Diese Methoden stören die Refraktion in den resultierenden Flüssigkeiten nicht.

Zentrifugieren, Koagulierung des Kaseins mit minimalen Mengen von Lab und Ultrafiltration wurden zu diesem Zwecke angewandt. Die Differenz zwischen den Brechungsindices der resultierenden Flüssigkeiten stellt den Beitrag der durch die Behandlung entfernten Bestandteile fest.

Eiweiss, Laktose und weniger bedeutenden Bestandteile tragen in dieser Reihenfolge zur Gesamtrefraktion der Milch bei. (Fett hat hierbei keinen Anteil). Im Verhältnis zu den in Milch vorhandenen Mengen haben Lactalbumin und Lactoglobulin den grössten Anteil, der grösser ist als der von Kasein.

Ein Kontrollexperiment wurde ausgeführt, um SCHULZE's Beobachtung, dass der Brechungsindex von Milch eine Anweisung für ihren Zuckergehalt ist, zu verifizieren.

Milchsera, die durch Behandlung mit Kupfersulfatlösung oder Lab als Präzipitantia erhalten wurden, zeigen, dass zwischen dem Brechungsindex und Laktosegehalt von Milch kein linearer Zusammenhang besteht.

Die Resultate aller obengenannten Experimente zeigen, dass die grosse Variabilität des Brechungsindex von Milch hauptsächlich durch die Veränderungen ihres Eiweissgehaltes verursacht wird.

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