rate of 1.8 IV units/min for hydrogenation at 180 C, 1,100 psig and 0.5% catalyst concentration, compared with 1.9, 2.0 and 1.8 obtained experimentally (Fig. 6).

DISCUSSION

One object of our study was to design a continuous slurry reactor capable of operating at high pressures (up to 5,000 psig and to determine the feasibility of its operation. At 180 C, 1,100 psig and 0.5 L/hr oil flow, the IV dropped 6 and 10 units for 0.5 and 1% catalyst; linolenic acid decreased to 4 and 2%. By increasing the temperature to 200 C, the IV drop increased to 12-16 units. Although this amount of IV drop is sufficient for salad oil production because the linolenate content dropped to 1%, further hydrogenation is necessary for the production of shortening and cooking oil. This was accomplished at the higher pressure. Iodine values of hydrogenated products were 17-43 units less than the original oil, depending on temperature and catalyst concentration. Therefore, any product within this range could be prepared by adjusting the parameters.

An attempt was made to compare continuous hydrogenation with previous results (3,7) of batch operation under similar conditions. Making exact comparisons is difficult because of slight differences in temperature, pressure and catalyst concentration and because of different lots of soybean oil used. The calculated residence times for the oil-catalyst slurry in the reactor were 4.5 min, 3.5 min and 2.4 min for 0.5 L/hr, 1.0 L/hr and 2.0 L/hr oil flow rates at 200 C and 1,100 psig. At 0.5 L/hr of oil flow, the decrease in IV was 11.7 units (Fig. 3), which compares favorably with batch hydrogenation. The residence times increased to 10, 6.2 and 3.5 min, respectively, for the 3 oil flow rates when pressure was increased to 4,500 psig. An IV drop of 23.5 units occurred at the higher pressure for a residence time of 4.5 min as read from the rate curve. Since this study was completed, another batch of oil was found to hydrogenate faster than the one used in our study. This increased activity probably was caused by better refining of the oil, which results in better removal of catalyst poisons. Copper catalysts have previously been shown to be sensitive to poisons in the oil (8). Selectivity at 1,100 psig was lower (8 vs 9) for continuous hydrogenation. But this high selectivity is still sufficient to reduce linolenic acid to less than 1% at 20 IV drop. No significant differences in isomerization were noted.

Our studies have shown that preparing salad oil as well as shortening and cooking oils is possible in a continuous system by changing hydrogenation conditions. Temperature, pressure, catalyst concentration and oil flow rate had no significant practical effect on selectivity or isomerization, but the hydrogenation rate generally increased with increase in temperature, pressure and catalyst concentration. To hydrogenate large volumes of vegetable oils at high pressure to a given IV, continuous reaction with selective copper chromite catalyst may offer economic advantages over batch hydrogenation.

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[Received October 22, 1983]

Detection of Cow Milk Admixture to Buffalo Milk

R.S. FARAG, Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt, **M.M. HEWEDI**, Food Industries Department, Faculty of Agriculture, El-Fayoum University, Egypt, **S.H. ABO-RAYA**, Food Science and Technology Department, Faculty of Agriculture, Cairo University, Egypt and **H.H. KHALIFA**, Animal Production Department, Faculty of Agriculture, Al-Azhar University, Egypt

ABSTRACT

A method has been devised that gives the distribution of various fatty acids of pure and adulterated buffalo milk with cow milk. Gas chromatography (GC) was used for the qualitative and quantitative determination of fatty acids of authentic buffalo milk, cow milk and buffalo milk adulterated with cow milk. The milk fat was separated by fractional crystallization at -20 C into 2 fractions, i.e., semisolid and mother liquor. The concentration of fatty acids in the mother liquor changed significantly for 14:0, 16:0 and 18:1 as adulteration levels were increased. The fatty acids of the semisolid fractions change in the proportion of 16:0, 18:0 and 18:1 when cow milk is mixed with buffalo milk. By applying simple regression equations for these acids, adulteration of buffalo milk with 5% cow milk could be distinguished.

INTRODUCTION

Buffalo milk is considerably higher priced than cow milk in Egypt. For economic as well as ethical reasons, therefore, ascertaining that buffalo milk offered for sale is free from adulteration by cow milk is desirable. Hence, a test is needed to check milk adulteration. This problem has been approached by analyzing fatty acids (1,2), unsaponifiables (3) and the ratios of some members of these lipid classes. Examining milk-protein fractions (4,5) also provides a sensitive method to detect adulteration. The present study was carried out to determine whether fatty acid analysis under certain conditions could be used as a decisive test to detect and determine the extent of adulteration of buffalo milk with cow milk.

MATERIALS AND METHODS

Milk Samples

Milk samples were collected during the winter from the Experimental Station Herd, Faculty of Agriculture, Cairo University, Giza, Egypt. The herd is comprised of Egyptian buffalos and Balady cows (30 each), 4-6 years old, that were milked twice daily. The animals were mainly fed Egyptian clover and concentrate mixtures during the winter season. Daily milk yield (morning and evening milking) was

thoroughly mixed and representative samples were taken. Three lots (5 kg each) from the representative milk samples were used to make 3 sets of cow and buffalo milk admixtures. Mixtures were prepared containing 5%, 10%, 15%, 20%, 25% and 30% (v/v) of cow milk in buffalo milk. These samples can be considered as authentic materials.

Preparation, Extraction and Fractionation of Lipid Materials

The milk samples were freeze-dried and the lipids were extracted with chloroform/methanol (2:1, v/v) (6). The lipids were then dissolved in silver nitrate-saturated methanol/acetone (70:30, v/v) and held at -20 C for 24 hr before the precipitate was filtered off. The ratio of lipid to silver nitrate-saturated solvent was 1:10 (w/v). The semisolid fraction was washed twice with the solvent precooled to -20 C. Thus, each lipid sample was separated by this technique into 2 fractions, i.e., semisolid and mother liquor. Sources of fatty acids, methanolysis and determination of fatty acids by gas liquid chromatography (GLC) were as described by Farag et al. (2) and Litchfield (7).

Statistical Analyses

The fatty acid percentages that changed the most be cause of adulteration with cow milk were subjected to various statistical analyses (Student's *t*-test, correlation coefficient r, coefficient of determination (R^2%), regression and multiple regression). The fatty acid percentages were transformed using arcsine transformation for correlation analysis (8).

RESULTS AND DISCUSSION

Fractional crystallization at -20 C was used to separate milk lipids into 2 distinct fractions. The separation depends on the degree of unsaturation (DU). The DU values can be deduced by applying the following equation: 1 (% monoenes/100) + 2 (% dienes/100). The DU values ranged from 0.53-0.69 and 0.20-0.26 in the mother liquor and semisolid fractions. GLC was used for the qualitative and quantitative determination of the various fatty acids of cow milk, buffalo milk and mixtures of the two. The chromatographic separation conditions were chosen to determine certain fatty acids (C_8-C_{18}).

Fatty Acid Composition of the Semisolid Fraction

Table I shows the fatty acid composition of lipids crystallized at -20 C for native cow and buffalo milk. Both milk sources contained 8:0, 15:0, 16:1, 17:0 and 18:2 as trace substances (<1%); 12:0 as a minor component (>1%-<10%) and 14:0, 16:0, 18:0 and 18:1 as major constituents (>10%). The most prevalent saturated and unsaturated acids were 16:0 and 18:1, respectively. The greatest differences in the fatty acid composition of cow and buffalo milk were in 16:0, 18:0 and 18:1 acids. For instance, buffalo milk contained 1.3 times as much 16:0 as that of cow milk. The amounts of 18:0 in cow milk were ca. 1.2 and 1.3 times greater than that in buffalo milk. These differences might be used as a criteria to characterize the milk origin.

Fatty Acid Composition of the Mother Liquor

The mother liquor obtained after cooling the whole-milk lipids at -20 C contained a wide range of fatty acids. Table II shows the fatty acid composition of cow and buffalo mother liquor as identified by gas chromatograph (GC). The mother liquor of both cow and buffalo milk contained traces of 8:0, 10:1, 11:0, 12:1, 13:1, 15:0, 15:1, 17:0 and 17:1; small percentages of 10:0, 12:0, 14:0, 14:1, 16:1 18:0 and 18:2 and large percentages of 16:0 and 18:1. They differed in their proportions. For example, the mother liquor of cow milk, compared with buffalo milk, contained more 14:0 and 16:0 acids. On the other hand, buffalo mother liquor had 16:1 and 18:1 acids ca. 2.1 and 1.4 times as great as that in cow mother liquor.

Determination of Buffalo Milk Adulteration

Adulteration of buffalo milk with cow milk can be detected by relying on certain fatty acids, e.g., 14:0, 16:0, 18:0 and 18:1. In the mother liquor, 14:0 and 16:0 increased significantly in concentrations at the 5% level of adulteration. No further increase was observed at higher adulteration levels. Conversely, a significant decrease in 18:1 was found at the 5% level, and this decrease was not altered at other adulteration levels. The fatty acid profiles of the semisolid fraction exhibited the following significant changes: a decrease in the concentrations of 16:0 and an increase in the quantities of 18:0 and 18:1. A considerable increase occurred in 8:0, 10:0 and 12:0, caused by mixing buffalo milk with cow milk. However, these acids were present in amounts less than 1% and any slight inaccuracy in measuring their amounts might lead to a mistaken conclusion.

The admixture of cow milk with buffalo milk can be checked quickly by applying simple regression equations

TABLE I

Composition (Percentage) of the Semisolid Fatty Acids Obtained from Buffalo Milk, Cow Milk and Adulterated Samples

Fatty acid	Buffalo milk	Extent of adulteration						Cow milk
		5%	10%	15%	20%	25%	30%	
8:0	0.3	0.4	0.4	0.7	0.7	0.9	1.0	0.9
10:0	0.5	0.6	1.0	0.7	0.8	1.0	1.0	1.0
12:0	1.4	1.8	1.5	1.6	2.1	1.9	1.9	2.8
14:0	12.8	12.7	11.5	12.0	12.5	12.4	12.0	12.8
14:1	0.4	0.5	0.3	0.6	0.9	1.0	1.0	1.0
15:0	0.2	0.5	0.2	0.4	0.6	0.7	0.6	0.7
16:0	51.0	47.5[a]	46.4[b]	46.0[b]	43.2[b]	43.6[b]	43.2[b]	38.9
16:1	0.6	1.0	1.0	0.8	0.7	0.9	0.6	0.5
17:0	0.1	0.1	0.2	0.4	0.4	0.7	0.6	0.6
18:0	14.0	14.8[a]	16.0[b]	16.2[b]	16.8[b]	16.4[b]	16.8[b]	17.3
18:1	18.3	20.0[b]	20.8[b]	20.2[b]	20.9[b]	20.0[b]	20.8[b]	23.0
18:2	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.5
DU[c]	0.20	0.22	0.23	0.22	0.23	0.23	0.23	0.26

[a]Mixtures significantly different from the native buffalo's milk at the 5% level.
[b]Mixtures significantly different from the native buffalo's milk at the 1% level.
[c]The degree of unsaturation [1(% monoenes/100) + 2(% dienes/100)].

TABLE II

Composition (Percentage) of the Mother Liquor Fatty Acids Obtained from Buffalo Milk, Cow Milk and Adulterated Samples

Fatty acid	Buffalo milk	Extent of adulteration						Cow milk
		5%	10%	15%	20%	25%	30%	
8:0	0.7	0.7	0.6	0.4	0.5	0.6	0.5	0.9
10:0	1.8	2.0	1.9	1.8	1.7	1.9	1.9	3.5
10:1	0.1	0.4	0.2	0.3	0.2	0.2	0.2	0.5
11:0	0.1	0.6	0.4	0.5	0.3	0.2	0.2	0.4
12:0	2.0	2.5	2.1	1.8	1.4	1.9	1.6	2.6
12:1	0.1	0.3	0.1	0.2	0.2	0.1	0.1	0.3
13:0	0.1	0.3	0.1	0.2	0.2	0.1	0.1	0.3
13:1	0.1	0.3	0.1	0.1	0.2	0.1	0.1	0.1
14:0	7.5	9.8[b]	11.0[b]	11.4[b]	11.1[b]	11.9[b]	11.6[b]	12.4
14:1	2.0	2.3	2.5	2.5	2.4	2.3	1.9	2.2
15:0	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.5
15:1	0.2	0.4	0.5	0.6	0.1	0.1	0.2	0.2
16:0	17.8	21.6[b]	22.2[b]	22.4[b]	22.4[b]	22.8[b]	23.2[b]	25.2
16:1	3.8	3.6	3.4	3.4	3.3	3.0	3.1	1.8
17:0	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.3
17:1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
18:0	3.2	2.9	2.3	3.0	3.0	2.6	2.5	3.5
18:1	57.5	49.9[b]	50.0[b]	49.8[b]	49.9[b]	50.0[b]	50.0[b]	42.0
18:2	2.7	2.0	2.3	2.1	2.5	2.9	2.8	3.0
DU[a]	0.69	0.62	0.62	0.62	0.62	0.62	0.61	0.53

[a]Indicates the degree of unsaturation.
[b]Mixtures significantly different from the native buffalo's milk at the 1% level.

TABLE III

Linear Regression Equations for the Adulteration Ratio (x) of Cow Milk Mixed with Native Buffalo Milk and Certain Fatty Acids (y)

Simple linear equations	R^2%[a]	r[b]	Multiple linear equations	R^2%[a]	r[b]
			Semisolid		
16:0 (y_1) = 47.487 − 0.098x	74.3	-0.86[c]	$x = -21.561 - 3.943y_1 + 10.953y_3$	77.7	0.88[c]
18:0 (y_2) = 15.420 + 0.024x	47.5	0.68[d]	$x = -396.47 - 3.525y_2 + 23.347y_3$	74.8	0.87[c]
18:1 (y_3) = 19.586 + 0.036x	74.3	0.86[c]	$x = 1153.303 - 15.394y_1 - 27.146y_2$	85.5	0.92[c]
			Mother liquor fatty acids		
14:0 (y_1) = 9.999 + 0.030x	36.4	0.603	$x = 451.731 - 3.945y_1 - 7.690y_3$	73.9	0.86[c]
16:0 (y_2) = 20.926 + 0.050x	57.5	0.76[d]	$x = -309.478 - 16.152y_1 + 22.925y_2$	65.8	0.81[c]
18:1 (y_3) = 52.743 − 0.111x	72.5	-0.85[c]	$x = 672.623 - 7.028y_2 - 9.842y_3$	74.7	0.86[c]

[a]Coefficient determination.
[b]Correlation coefficient.
[c]Significance at the 5% level.
[d]Significance at the 1% level.

for certain fatty acids (Table III). By analyzing the milk fatty acids and introducing their percentages in the linear equations, the milk adulteration can be characterized. The correlation-coefficient data shows that the percentage of 18:1 in the semisolid fraction can be used as a criterion to detect milk admixture, from the 5% level and up. However, the percentage of adulteration cannot be determined. The multiple-regression equation for 16:0 and 18:0 is the most helpful in detecting the extent of adulteration because the R^2 value was 85.5%. To measure the extent of adulteration with mother liquor fatty acids, 18:1 is the most helpful because it showed the highest R^2 value (75.5%). The other multiple-regression equations did not add further information relevant to the different adulteration levels. Thus, the equations for 16:0 and 18:1 are preferable for characterizing adulteration of buffalo milk (R^2 = 74.7%). Generally, equations for the semisolid fatty acids are far more sensitive than the equations for the mother liquor fatty acids in detecting milk adulteration.

The structure of milk fatty acids depends to some extent on the type of diet used. Also, significant variations in the physico-chemical characteristics of butter were found between seasons and between provinces (9). Consequently, the present method provides a basis for the detection of milk adulteration only in Giza governorate. In order to generalize the present technique, the analyst should analyse the milk fatty acids at different seasons and at various locations to deduce the regression equations. In other words, every district should have its own regression equations for certain fatty acids to characterize milk adulteration.

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[Received September 19, 1983]

Interaction of Proteins with Sorghum Tannin: Mechanism, Specificity and Significance

LARRY G. BUTLER, DAVID J. RIEDL, D.G. LEBRYK and **H.J. BLYTT,** Department of Biochemistry, Purdue University, West Lafayette, IN 47907

ABSTRACT

The grain of some varieties of sorghum contains 2% or more condensed tannin; many other varieties contain no tannin at all. Agronomic advantages, e.g., resistance to bird depredation, are associated with high-tannin sorghums, which have relatively low nutritional value for nonruminants. The biological effects of tannin are a result of its propensity for binding proteins; both hydrogen bonding and hydrophobic interactions are involved. Sorghum tannins can bind dietary proteins and reduce their digestibility. Purified digestive enzymes are inhibited by tannin, but significant inhibition in vivo is unlikely. Proteins differ greatly in their affinity for tannin. Those with highest affinity are large, have an open structure, contain no bound carbohydrate and are rich in proline. Sorghum proteins of the alcohol-soluble prolamine fraction associate strongly with tannin, are difficult to remove during tannin purification and are found combined with tannin in the indigestible residue after in vitro digestion with pepsin. On germination, the seed may sacrifice a portion of these proteins to bind the tannin that might otherwise interfere with metabolism by inhibiting seed enzymes. During seed development, tannin molecules are relatively short and do not effectively precipitate proteins; as the seed dries, tannins undergo polymerization to an average of ca. 6 flavan-3-ol units/molecule. The antinutritional effects of sorghum tannins can be eliminated by soaking the grain in dilute aqueous alkali, but not by cooking. When rats are put on high-tannin sorghum diets, their parotid glands undergo hypertrophy and produce a group of unique salivary proteins with extremely high affinity for tannin. These proteins contain over 40% proline and are devoid of sulfur-containing and aromatic amino acids. This metabolic adaption may protect rats against tannin by binding and inactivating it immediately when it enters the digestive tract.

INTRODUCTION

Tannins are plant secondary substances (not in metabolic pathways providing energy for growth and reproduction) that are characteristically rich in phenolic hydroxyl groups (1). Tannins exhibit a wide variety of biological effects thought to be caused by their capacity to bind and coagulate proteinaceous tissue (astringency) (2). Indeed, the name "tannin" is from their historically important use in tanning hides into leather by binding proteins such as collagen in animal skins.

Both major structural classes of tannins are widely distributed in plants (1). Hydrolyzable tannins are phenolic carboxylic acids, e.g., gallic acid, esterified to sugars such as glucose. Condensed (nonhydrolzable) tannins, chemically known as proanthocyanidins, are polymers of flavin-3-ols linked by carbon-carbon bonds.

Mature grain of the important cereal *Sorghum bicolor* (L.) Moench may contain up to 2% or more condensed tannin, although many lines contain no tannin at all; immature grain of high-tannin sorghums shows even higher levels of tannin in chemical assays (3). Hydrolyzable tannins have not been reported from sorghum. Under optimal conditions, sorghum tannin is capable of binding and precipitating at least 12 times its own weight of protein (4). Because sorghum grain contains ca. 10% protein (5), the grain of high-tannin cultivars contains more than enough tannin to bind all the seed protein, thus profoundly affecting the properties of the protein. Other tannin-containing crops, e.g., barley, rye and common beans, contain lower amounts of tannin and higher levels of protein (6); the protein of these crops is less affected by the presence of tannin.

The purpose of this article is to review the current knowledge of the mechanism, specificity and significance of the interaction of sorghum tannins with proteins.

MECHANISM

Proteins have been shown to interact with tannins by means of hydrogen bonding (12), hydrophobic interaction (13), electrostatic attraction (14) and covalent bonding associated with oxidation (15).

Electrostatic attraction does not apply to the sorghum tannin-protein system. The only ionizing groups of condensed tannins are phenolic hydroxyls; in order to ionize these groups the pH would be so high that most proteins would have similar negative charges, so any electrostatic interaction would be repulsion rather than attraction. The precipitation of soluble proteins by tannin is maximal near the isoelectric point of the protein, when the net charge on the protein is zero (16). Proteins with high isoelectric points, e.g., egg white lysozyme, not only precipitate over a broad pH range (16), but also enhance coprecipitation of more acidic proteins at intermediate pH values, probably by electrostatic interaction between proteins of opposite net charge (17).

No evidence exists that sorghum tannins bind protein covalently, with the possible exception that purified sorghum tannin always contains ca. 2% (by weight) protein contaminant (18). Precipitates of protein with sorghum tannin (17), as well as protein complexes with other tannins

Presented at the AOCS Meeting, May 11, 1983.

Journal Paper Number 9552, Agricultural Experiment Station, Purdue University. Supported in part by USAID, Title XII, PRF-4B, and by NIH Grant GM23028.