

3. The optimum concentration of ammonium chloride for the growth of yeast is the concentration of the salt in which a protein (wheat gluten) is least swollen.

4. The optimum concentration of ammonium chloride varies with the temperature. At the temperatures tested the concentrations of the salt causing the least swelling of a protein (wheat gluten) are identical with those most favorable to yeast growth.

5. The effect of certain colloidal materials upon the growth of yeast has been determined.

AMES, IOWA.

[CONTRIBUTION FROM OIL, FAT AND WAX LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

AN ANALYSIS OF OTOBA BUTTER.

BY WALTER F. BAUGHMAN, GEORGE S. JAMIESON AND DIRK H. BRAUNS.

Received October 26, 1920.

A sample of otopa butter from Colombia, South America, was recently submitted to this laboratory for examination by Dr. David Fairchild, of the Office of Foreign Seed and Plant Introduction of the Department of Agriculture, and it is believed that the results of the examination are of sufficient importance to warrant making a permanent record of them.

Otopa butter is the common name for the fat expressed from the fruit of *Myristica otopa*. It is also sometimes called American nutmeg butter, American mace butter, and otopa wax. It has long been used by the people of Colombia, S. A., as a medicament for skin diseases of domestic animals.

E. Uricoechea¹ made a cursory examination of this product and reported that it resembled nutmeg butter, and consisted of the glycerides of myristic and oleic acids, with a considerable amount of unsaponifiable matter. From the unsaponifiable constituents he separated a crystalline compound, to which he gave the name "otobite" and the formula $C_{24}H_{26}O_5$. He makes no mention of a volatile oil.

Experimental Part.

Physical and Chemical Characteristics of the Fat.—The freshly extracted fat has been described as being white, having the consistency of butter, and an odor resembling that of nutmegs. The sample used in this investigation had acquired a brown color and a disagreeable odor, a change which had been noted by previous observers. The chemical and physical characteristics are given in Table I. It will be noticed that the butter is a mixture of a volatile or essential oil, a fixed oil and unsaponifiable matter.

¹ E. Uricoechea, *Ann.*, **91**, 369 (1854).

TABLE I.
Chemical and Physical Characteristics.

Specific gravity 20°/20°.....	0.9293
Refractive index, 40°.....	1.4710
Iodine number (Hanus).....	54.0
Saponification value.....	185.0
Melting point.....	34.0° C.
Essential oil.....	9.3%
Unsaponifiable matter (other than essential oil).....	20.4%
Fatty acids.....	67.6%

Essential Oil.—The essential oil, which is present to the extent of 9.3%, was separated by steam distillation. It has a pale yellow color, which fades on exposure to the light, and only a slight odor. It differs greatly in its character and composition from ordinary nutmeg butter, as will be seen by the following data: d_{20}^{20} , 0.89067; $[\alpha]_D^{20}$ —32°; and n_{20}^{20} , 1.4180. A 90-g. portion of the oil was subjected to fractional distillation under diminished pressure. The data of this distillation are given in Table II.

TABLE II.
Fractional Distillation *in Vacuo* of Essential Oil.

Fraction.	Pressure. Mm.	Temperature. °C.	Weight. G.
1.....	13	126–129	16.1
2.....	15	134–137	31.7
3.....	15	138–142	10.6
4.....	15	142–147	8.4
5.....	15	147–175	3.0
6.....	15	176–190	2.0
Residue.....	18.0
			—
			89.8

The residue from the distillation was a viscous, dark yellow mass which gradually became, in part, crystalline on standing. It probably consisted largely of polymerization products of the constituents of the essential oil.

An elementary analysis was made of each fraction, and also specific gravity, specific rotation and refractive index determinations were made. The results are given in Table III. The molecular weight of Fraction 2,

TABLE III.
Results of Analyses of Fractions Obtained by Distilling Essential Oil.

Fraction.	Carbon. %.	Hydrogen. %.	d_4^{20} .	$[\alpha]_D^{20}$.	Refractive index, 20°.
1.....	87.25	11.96	0.8935	—17.84	1.4881
2.....	87.46	11.69	0.9002	—20.71	1.4962
3.....	86.95	11.62	0.9054	—14.70	1.4952
4.....	86.99	11.67	0.9101	—0.90	1.4972
5.....	83.86	11.30	0.9351	+14.0	1.4970
6.....	77.06	11.48	0.9348	—6.9	..

determined by the freezing-point method, was found to be 204, which agrees with the molecular weight of a sesquiterpene, $C_{15}H_{24}$. All fractions gave the color test for the sesquiterpene cadinene, Fraction 5 giving the test most readily. This test was made by adding 2 drops of the fraction to 5 cc. of glacial acetic acid, followed by 2 drops of conc. sulfuric acid. A yellowish-green color appeared at once, but after a short time the characteristic deep violet color developed. It is believed that the essential oil consists largely of sesquiterpenes.

Unsaponifiable Constituents of the Fat.—The butter from which the essential oil had been removed was saponified with alcoholic potash. The resulting soap was dissolved in water, and the unsaponifiable matter extracted repeatedly with ether. After the removal of the solvent, the residue was dissolved in hot 95% alcohol. On cooling, the alcoholic solution deposited a copious crop of crystals in the form of white silky orthorhombic prisms. This is the otobite described by Uricoechea. It composes about 46% of the unsaponifiable matter, the remainder, after the removal of the alcohol, forming a very viscous yellow mass.

The crystalline material was recrystallized several times from absolute alcohol and a product obtained with a melting point of 137–138° which was not changed by further recrystallization. From the alcoholic, mother liquors another product was obtained which crystallized in small, needle-like prisms. When purified as completely as possible it melted at 106–108°.

The otobite described by Uricoechea melted at 135°. He makes no mention of the occurrence of a second similar substance, so it is evident that the compound which he obtained was a mixture of 2 compounds. For the compound with the higher melting point the name otobite may be retained and the other compound designated as iso-otobite.

The results of the analyses of these 2 compounds are given below:

Calc. for $C_{20}H_{30}O_4$: C, 74.05; H, 6.21; O, 19.74.

Otobite, found: C, 74.10; H, 6.19; O, 19.71; $[\alpha]_D^{20}$ -35.7 ; mol. wt., 310.

Iso-otobite, found: C, 74.29; H, 6.06; O, 19.64; $[\alpha]_D^{20}$ $+5.3$; mol. wt., 315.

A small amount of either otobite, when added to conc. sulfuric acid, produces a pink color which becomes very strong on standing and remains unchanged for days. If several drops of conc. sulfuric acid are added to a glacial acetic acid solution of either compound (phytosterol test) a pink color gradually develops, which, after some hours, becomes somewhat purple and remains unchanged for several days. This is in marked contrast to the changing colors produced by phytosterols. Both compounds were boiled for several hours with acetic anhydride in the presence of anhydrous sodium acetate, but no reaction took place and the unaltered compounds were recovered. Several unsuccessful attempts were made to methylate them with methyl sulfate. They were not altered

when fused with caustic potash. Strong, alcoholic potash was also without effect.

The Zeisel method for the determination of the methoxy group gave the following results.

Otobite, 0.2231, 0.2184: AgI, 0.1425, 0.1380.

Calc. for $C_{20}H_{20}O_4$: CH_3O , 9.56. Found: 8.43, 8.34.

From iso-otobite not a trace of AgI was obtained. Therefore, otobite contains one methoxy group and iso-otobite none.

In an attempt to make the bromo derivative of otobite, 2 g. of otobite was dissolved in 75 cc. of absolute ether. The solution was cooled to 5° and 2 g. of bromine slowly added while the flask was gently shaken. It was observed that a small amount of hydrobromic acid was formed.

After standing for 2 hours the excess of bromine was removed by washing with an aqueous solution of sodium thiosulfate. The ether solution was then dried over anhydrous sodium sulfate, after which about $\frac{2}{3}$ of the ether was distilled off and the residual solution was allowed to remain in the ice-box overnight. The compound which crystallized out in flat monoclinic prisms had a very pale pink color which could not be removed by washing with chilled ether. It melted at 190° and contained 54.86% of bromine. The calculated bromine content of $C_{20}H_{20}O_4Br_5$ is 55.21%. It appears, therefore, that the compound is a pentabromide. It is very soluble in alcohol, acetone, ether and petroleum ether.

The bromo derivative of the iso-otobite was made in a similar manner, except that it was dissolved in glacial acetic acid instead of ether. The bromide obtained crystallized in flat, monoclinic prisms, melted at 191° C. and contained 54.77% bromine. It also appears to be a pentabromide. It is very soluble in ether, petroleum ether, acetone and chloroform.

Unsaturated Acids.—The iodine number of the original butter is not large and measures not only the iodine absorbed by the unsaturated acids, but also that assimilated by the unsaponifiable matter. It is evident, therefore, that the content of unsaturated acids is small. It was found that a satisfactory separation of the saturated acids from the small amount of unsaturated acids could not be effected by the lead salt-ether method. The bulk of the saturated acids was removed from 78 g. of the fatty acid mixture (free from unsaponifiable matter) by fractional crystallization from alcohol. The mother liquor which contained the unsaturated acids was heated with alcoholic potash and the excess of potash neutralized with acetic acid. A further separation of the saturated acids was effected by precipitating with an alcoholic solution of magnesium acetate. Finally, the unsaturated acids were separated by the lead salt-ether method. The weight of the unsaturated acid fraction thus obtained was 5.1 g. and the iodine number 75.5. This is less than the iodine number of oleic acid (90) and indicates that the saturated acids had not been completely removed. Upon brominating, no tetrabromide was obtained.

showing the absence of linolic acid. The percentage of oleic acid calculated from the iodine number of the unsaturated acid fraction is 5.5% of the fatty acids, or 3.7% of the original butter. This is equivalent to 3.9% of oleic acid glyceride.

Saturated Acids.—The fatty acids were recovered from the soap solution from which the unsaponifiable matter had been extracted and converted into methyl esters. The esters, weighing 250 g., were subjected to fractional distillation under diminished pressure which resulted in 8 fractions and a residue. Subsequently it was discovered that the residue and the higher boiling fractions contained some unsaponifiable matter. These fractions, therefore, were not suitable for quantitative analysis, but satisfactory for qualitative examination. The following acids were identified.

Lauric Acid, $C_{12}H_{24}O_2$, m. p. 44° .—The free acids were recovered from Fraction 1, which weighed 72 g. and recrystallized from 50% alcohol. A large fraction was obtained which melted at 44° . An elementary analysis gave the following results.

Calc. for lauric acid: C, 71.93; H, 12.06. Found: C, 71.97; H, 12.06.

Lauric acid was also obtained from Fraction 2.

Myristic Acid, $C_{12}H_{22}O_2$, m. p. 53.8° .—Fraction 2 weighed 115 g. Upon crystallizing the free acids from alcohol a large quantity of myristic acid melting at 54° was obtained.

Palmitic Acid, $C_{16}H_{32}O_2$, m. p. 62.6° .—The free acids from Fractions 6 and 7, which weighed 6 g. and 3 g., respectively, were recrystallized from alcohol and a product obtained which melted at 63° to 64° , and had the following elementary composition.

Calc. for palmitic acid: C, 74.92; H, 12.59. Found: C, 75.09; H, 12.52.

Thus the saturated acids present in the fatty acid mixture were identified. In order to make a quantitative examination another portion of the fatty acid mixture was freed with great care from the unsaponifiable matter, converted into the methyl esters and distilled under diminished pressure. The data of the distillation are given in Table IV. The results of the analyses of these fractions and the calculated amounts of the various acids present are recorded in Table V. The data in Table V are summed up in Table VI.

TABLE IV.
Fractional Distillation *in Vacuo* of Methyl Esters of Saturated Acids.
36.5 G. Esters Subjected to Distillation.

Fraction.	Temperature, ° C.	Pressure. Mm.	Weight. G.
1.....	138-140	5	8.0
2.....	141 quickly ran to 147, 147-150	5	22.7
3.....	151 quickly ran to 160, 160-163	5	4.2
Residue.....			1.6
			36.5

TABLE V.

Results of Analyses of Fractions Obtained by Distilling Methyl Esters of Saturated Acids.

Fraction.	Iodine number.	Saponification value.	Mean molecular wt.	Unsaturated esters of acids.		Mean molecular weight of esters of saturated acids.	Lauric acid.		Myristic acid.		Palmitic acid.	
				%.	G.		%.	G.	%.	G.	%.	G.
1.....	1.0	245.6	228.4	1.2	0.1	227.8	48.1	3.8	45.1	3.6
2.....	1.1	235.3	238.0	1.3	0.3	237.8	15.0	3.4	78.4	17.8
3.....	6.3	227.8	246.3	7.3	0.3	243.0	84.7	3.6	3.1	0.1
Residue	46.0	242.1
				Total,		0.7	7.2		25.0		0.1	

TABLE VI.

Composition of Saturated Acid Mixture.

	G.	%.	Original oil. %.	Glycerides in original oil. %.
Lauric acid.....	7.2	22.3	14.2	15.1
Myristic acid.....	25.0	77.4	49.5	52.2
Palmitic acid.....	0.1	0.3	0.2	0.2
Total.....	32.3	100.0	63.9	67.5

Summary.

The chemical and physical characteristics of a sample of otoba butter have been determined. The glyceride of lauric acid and iso-otobite have not been previously reported as occurring in otoba butter.

Its chemical composition has been studied and found to be as follows:

Glycerides of	{	Lauric acid.....	15.1
		Myristic acid.....	52.2
		Palmitic acid.....	0.2
		Oleic acid.....	3.9
Essential oil, chiefly sesquiterpenes.....		9.3	
Unsaponifiable constituents	{	Otobite and iso-otobite (C ₂₀ H ₂₀ O ₄) ..	9.4
		The viscous mass.....	11.0

We are indebted to Dr. F. B. Power, of this Bureau, for many helpful suggestions in regard to the proper procedure to be followed in making this investigation.