Characterization of Spanish Orange Juice for Variables Used in Purity Control

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Orange juice concentrates (147 samples) obtained in six Spanish citrus plants during two crop seasons from the most important groups of orange varieties grown in Spain were characterized for 28 analytical variables, all of them used to control juice purity. Due to seasonal and varietal differences (significant at the 99% level), the whole set of 147 samples cannot be considered a unique reference population to detect orange juice dilutions. When doing this, poor detection effectiveness of adulterated samples and erroneous rejection of too many pure juices (9%) were observed. Both problems were avoided by considering independent populations corresponding to the different seasons and groups of varieties.

Orange juices consumed in the European community come from different sources (several Mediterranean countries as Greece, Italy, Morocco, and Spain or other countries further from Europe such as Brazil). Geographical and varietal differences as well as farming practices cause great variability in the analytical characteristics of the juice, some of them used to control purity. As purity control methods are generally based on the comparison of unknown samples with data acquired from pure juices, knowledge of the characteristics of juices from all sources is essential. Available data of Spanish juices mainly consist of mineral contents (Primo and Royo, 1965, 1967; Royo and Giménez, 1974), but there is not enough information about other characteristics presently used in purity control, as Navarro and Izquierdo (1988) recently pointed out. The purpose of this paper is to offer data obtained from Spanish pure juices in which amino acids, sugars, minerals, ash, citric and isocitric acid, and spectral characteristics were determined. Variability of data and differences due to season and orange variety are discussed. Suitability of obtained data to build reference sets of pure juices to detect dilutions is also discussed.

MATERIALS AND METHODS

Raw Material. Orange concentrates of 65° Brix (147 samples) obtained from White (Spanish denomination for nonblood, nonnavel orange varieties such as Salustiana, Comuna, Cadenera, Valencia, Verna, etc.) and Navel oranges were collected in six Spanish citrus plants from December to May during two crop seasons (1983–1984, 1984–1985). Concentrates were stored at −18 °C. All analyses were performed on juices reconstituted to 11° Brix with redistilled water.

Chemical and Instrumental Analyses. *Acids.* Titratable acids (total acids) were determined by direct titration of a 10-mL aliquot to pH 8.1. Results were expressed as citric acid in accordance with the method reported by the International Federation of Fruit Juice Producers (1968). D-Isocitric acid was enzymatically determined following the method of Boehringer Mannheim Biochemicals (1980).

Sugars. Sucrose, glucose, and fructose were determined by HPLC in a Perkin-Elmer Series 2 liquid chromatograph (using a Shodex S-801/S column, Showa Denko, K.K., Japan) thermostated at 60 °C and a Perkin-Elmer refractive index detector, Model LC25. The samples were prepared by percolating 1 mL of orange juice and 1 mL of rafinose solution (internal standard, 3.5 mg/100 mL) through a SepPak C18 (Waters Española, S.A., Barcelona) and by washing with 3 mL of water. An aliquot was used for HPLC analysis after filtering through a 0.22-μm filter (Millipore S.A., Catalog No. GSTF01300).

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Table I. Mean Values[a] and Dispersions of 147 Spanish Orange Juices

	variable	mean	coeff of variation, %	range of values min	range of values max
1	aspartic acid	16.9	47.1	2.7	49.4
2	glutamic acid	9.11	39.3	1.9	21.9
3	asparagine	45.7	35.1	11.3	93.3
4	glutamine	3.50	45.4	0.9	8.8
5	serine	12.8	34.3	4.4	29.7
6	threonine	2.13	36.6	0.7	5.0
7	glycine	2.72	21.6	1.2	5.8
8	alanine	8.80	30.5	3.4	18.3
9	arginine	41.4	43.1	8.9	95.3
10	γ-aminobutyric acid	23.7	33.3	6.2	47.0
11	proline	162.0	27.4	61.8	397.0
12	valine	1.53	46.4	0.3	6.2
13	methionine	1.26	78.8	0.1	4.2
14	ornithine	1.20	68.8	0.3	9.7
15	lysine	3.05	44.3	0.5	6.3
16	histidine	0.86	55.3	0.3	4.3
17	abs 280 nm	1.44	31.8	0.4	3.1
18	abs 325 nm	0.94	39.2	0.3	2.2
19	abs 443 nm	0.12	57.0	0.0	0.5
20	potassium	142.0	14.1	77.7	187.0
21	magnesium	9.90	10.7	8.1	14.7
22	calcium	11.4	36.4	5.4	21.2
23	ash	322.0	8.9	251.0	425.0
24	isocitric acid	11.5	27.7	2.2	20.7
25	citric acid	1090.0	25.7	509.0	2070.0
26	sucrose	3810.0	10.3	2900.0	4760.0
27	glucose	2080.0	12.0	1320.0	2920.0
28	fructose	2460.0	12.1	1550.0	3350.0

[a] All values are expressed as milligrams/100 mL with the exception of absorbances.

Amino Acids. Individual free amino acids were determined as dansyl derivatives by HPLC (Navarro et al., 1984).

Spectral Characteristics. The visible (443 nm) and ultraviolet (325, 280 nm) absorption of samples was measured by the method described by Petrus and Attaway (1980).

Minerals. Potassium, magnesium, and calcium were determined by atomic absorption (McHard et al., 1976).

Ash. It was determined following the method proposed by the Association of Official Analytical Chemists (1980).

Statistics. Data analysis was accomplished by using BMDP statistical software (Los Angeles, CA).

RESULTS AND DISCUSSION

Data Characteristics. Table I shows the average values of the 28 analytical characteristics determined in the 147 juices, as well as the standard deviations and the coefficients of variation.

As can be observed in Table I, the dispersion is high for almost all variables. The coefficients of variation of amino

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Table II. Correlation Coefficients between Variables

		1	2	3	4	5	6	7	8	9	10	11	12	13	15	17	18	19	21	22	27	28
1	aspartic acid	1.00	0.88	0.60		0.54																
2	glutamic acid		1.00	0.62		0.61	0.57			0.57												
3	asparagine			1.00	0.75	0.88	0.82			0.82												
4	glutamine				1.00	0.79	0.73	0.51		0.78					0.54							
5	serine					1.00	0.87		0.65	0.85					0.57							
6	threonine						1.00	0.56	0.56	0.83			0.55									
7	glycine							1.00				0.64										
8	alanine								1.00		0.70				0.69							
9	arginine									1.00					0.55							
10	γ-aminobutyric acid										1.00				0.53							
11	proline											1.00	0.56									
12	valine												1.00									
13	methionine													1.00	0.60					0.65		
15	lysine														1.00					0.52		
17	abs 280 nm															1.00	0.92	0.82				
18	abs 325 nm																1.00	0.73		0.52		
19	abs 440 nm																	1.00				
21	magnesium																		1.00	0.69		
22	calcium																			1.00		
27	glucose																				1.00	0.90
28	fructose																					1.00

[a] Correlation coefficients from −0.5 to +0.5 are not shown. Negative correlation coefficients lower than 0.5 were not observed.

acids ranged from 22 to 79%, but only in the cases of glycine and proline were the coefficients lower than 30%. Most of the coefficients ranged from 30 to 50%. Histidine showed a coefficient of variation of 55%, ornithine 69%, and methionine 79%. Isocitric acid and total acids showed similar coefficients of variation (28 and 26%), whereas the dispersion of spectral characteristics increased (from 32 to 57%) as wavelength did. The lowest dispersions were observed for minerals and sugars with coefficients from 9 to 14% except for calicum (36%).

Table II shows those correlation coefficients higher than 0.5 between variables. It must be noted that these coefficients were observed almost exclusively between variables of the same nature, i.e. amino acids, sugars, or minerals, whereas between variables of different nature the coefficients were generally lower than 0.5 (not shown in the table). The only exception was calcium, showing a correlation coefficient of 0.65 with methionine, of 0.52 with lysine, and of 0.52 with the absorption at 325 nm. The value 0.5 was arbitrarily chosen to reduce the entries of Table II.

Detecting Adulterations Using the Whole Set of Data. A common way of orange juice adulteration is dilution with water, masked by the addition of sugar and citric acid. As a consequence, the concentrations of unmasked constituents decrease in diluted juices. These concentrations can be compared with those of a reference population of natural juices to detect the dilution. Among multivariate statistical methods used to carry out comparisons between a juice and a reference population, the χ^2 test on the Mahalanobis distance is often used (Lifshitz et al., 1974; Ooghe and Waele, 1982; Cohen et al., 1984; Brown et al., 1988). Mahalanobis distance is calculated by

$$D^2 = \sum_{i=1}^{m} \sum_{j=i}^{m} (x_i - \mu_i)\, b_{ij} (x_j - \mu_j)$$

where D^2 is the square of the Mahalanobis distance, m is the number of constituents or characteristics (variables) considered ($i = 1, ..., i, ..., m, j = 1, ..., j, ..., m$), x_i and x_j are their values in the juice tested, μ_i and μ_j are the mean values in the reference population of natural juices, and b_{ij} are the elments of the inverse of the covariance matrix obtained from this population.

The square of the Mahalanobis distance (D^2) of juices belonging to the reference population is distributed as a χ^2 variable with m degrees of freedom. So, if D^2 is higher than the χ^2 value at a given confidence level, it may be well supposed that the juice does not belong to the reference population.

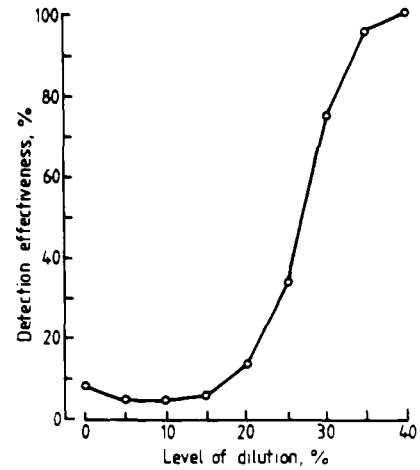

Figure 1. Effectiveness in detecting dilutions when using all juices as a unique reference population.

Considering the whole set of 147 juices of this work as a reference population and each one of the juices diluted at different levels (computer simulation) as adulterated samples, the results shown in Figure 1 were obtained. The figure represents, in function of the dilution level, the percentage of diluted samples detected, that is, the percentage of juices for which D^2 was higher than the χ^2 value. The selected confidence level was 99%. Sucrose, fructose, glucose, and total acids were not considered as some or all of them are supposed to have been added to mask the dilution. Thus, the Mahalanobis distance was calculated on the basis of 24 variables (variables 1–24 of Table I).

Two important conclusions can be obtained from Figure 1: Detection effectiveness of diluted samples was poor (25% of dilution was detected only in 34% of the cases), and erroneous rejection of pure samples was high (9%) when the whole set of 147 samples was used as a unique reference population of pure juices.

This is not surprising when the characteristics of the reference set are considered. The samples were collected during two crop seasons from two groups of varieties. Since varietal and seasonal differences existed, as will be discussed in the next section, the whole set cannot be

Table III. Mean Values in Relation to Variety and Crop Season

		variety[a]			season			variety means/season				
	variable	White (95)	Navel (52)	diff	first (83)	second (64)	diff	White, first season (46)	White, second season (49)	Navel, first season (37)	Navel, second season (15)	interaction
1	aspartic acid	16.8	17.1		18.8	14.4	*b*	19.5	14.3	18.0	14.9	
2	glutamic acid	9.17	9.00		9.61	8.47		10.2	8.22	8.90	9.25	
3	asparagine	44.4	48.1		47.0	44.0		47.8	41.2	46.0	53.2	
4	glutamine	3.50	3.52		3.11	4.02	*b*	3.25	3.74	2.94	4.93	
5	serine	13.0	12.5		12.0	13.8	*b*	12.6	13.3	11.2	15.6	
6	threonine	2.10	2.20		2.05	2.24		2.10	2.09	1.99	2.70	
7	glycine	2.76	2.66		2.63	2.84		2.72	2.80	2.52	2.98	
8	alanine	9.23	8.03		7.99	9.86	*b*	8.54	9.88	7.30	9.81	
9	arginine	39.1	45.6		39.1	44.3		39.1	39.1	39.1	61.4	*b*
10	γ-aminobutyric acid	25.5	20.6	*b*	22.9	24.9		25.1	25.8	20.1	21.8	
11	proline	162.0	161.0		151.0	176.0	*b*	150.0	173.0	151.0	185.0	
12	valine	1.59	1.45		1.38	1.76	*b*	1.44	1.73	1.30	1.81	
13	methionine	1.38	1.06		0.57	2.17	*b*	0.57	2.16	0.57	2.26	
14	ornithine	1.14	1.33	*b*	1.17	1.25		1.05	1.03	1.31	1.36	
15	lysine	3.17	2.82		2.37	3.94	*b*	2.56	3.76	2.14	4.51	
16	histidine	0.88	0.85		0.82	0.93		0.82	0.93	0.82	0.91	
17	abs 280 nm	1.48	1.38		1.26	1.69	*b*	1.21	1.74	1.32	1.54	
18	abs 325 nm	1.01	0.83		0.76	1.19	*b*	0.78	1.22	0.73	1.04	
19	abs 443 nm	0.13	0.12		0.10	0.16	*b*	0.09	0.17	0.11	0.13	
20	potassium	140.0	145.0		152.0	129.0	*b*	153.0	127.0	149.0	134.0	
21	magnesium	9.95	9.83		9.39	10.6	*b*	9.24	10.6	9.58	10.4	
22	calcium	12.0	10.3	*b*	8.29	15.5	*b*	8.15	15.7	8.46	10.5	
23	ash	326.0	315.0		318.0	328.0		320.0	332.0	315.0	315.0	
24	isocitric acid	11.9	10.7		11.5	11.4		12.1	11.7	10.7	10.5	
25	citric acid	1140.0	1010.0		1030.0	1190.0	*b*	1070.0	1220.0	980.0	1090.0	
26	sucrose	3810.0	3790.0		3780.0	3840.0		3760.0	3860.0	3810.0	3780.0	
27	glucose	2090.0	2070.0		2000.0	2190.0	*b*	1980.0	2200.0	2030.0	2150.0	
28	fructose	2480.0	2440.0		2360.0	2600.0	*b*	2300.0	2630.0	2400.0	2510.0	

[a] Number of samples in parentheses. [b] Difference or the interaction was significant at the 99% level.

Table IV. Classification of Juices by Discriminant Analysis

	actual			
classificn	White oranges, first season	Navel oranges, first season	White oranges, second season	Navel oranges, second season
White oranges, first season	36	6	0	0
Navel oranges, first season	10	31	0	0
White oranges, second season	0	0	40	3
Navel oranges, second season	0	0	9	12

considered as a unique population and, therefore, Mahalanobis theory, based on the distance from a sample to a unique population, cannot be strictly applied.

Varietal and Seasonal Differences. When the 147 samples are grouped in the varietal and seasonal groups they belong to, the mean values shown in Table III were obtained. The number of samples constituting each group is also indicated in the table as well as the significance of the differences between mean values for each variable at the 99% confidence level. Significant differences between varieties were only found for γ-aminobutyric acid, ornithine, and calcium, suggesting that the variables considered in this work are not very efficient to characterize orange varieties. This aspect validates the utility of the selected variables from the point of view of purity control, since purity, not varietal origin, is being tested.

On the contrary, seasonal differences were high. Of the 28 variables, 17 showed significant differences at the 99% level between the two seasons.

Season–variety interaction was not significant for any variable except arginine, due to its high value in Navel oranges from the second season. Multivariate analysis of variance leads to the canonical representation shown in Figure 2, thus confirming the results obtained by univariate analysis. A complete differentiation of samples, without any overlapping, is observed between crop seasons. Groups of varieties were also distinctly represented but with some overlapping, which shows that varietal differences were smaller than seasonal ones. This picture is quantified in Table IV where results of discriminant analysis are shown. All samples were correctly classified as belonging to their actual crop season whereas in relation to varietal source about 18–20% of incorrect classifications was observed.

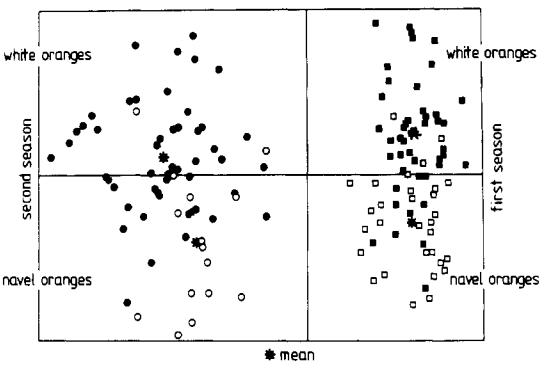

Figure 2. Representation of juices as a function of the first two canonical variables obtained by multivariate analysis of variance.

Detecting Adulterations Using Each Population as an Independent Set. Using Mahalanobis distance principle independently for each population (with the exception of Navel oranges from the second season, since the number of samples was lower than the number of variables), the results shown in Figure 3 were obtained. Mean

Table V. Rejection of Pure Juices (%) According to the Subpopulation Selected as Reference Set

ref subpopulation	subpopulation tested			
	White oranges, first season	Navel oranges, first season	White oranges, second season	Navel oranges, second season
White oranges, first season	0	84	100	100
Navel oranges, first season	87	0	100	100
White oranges, second season	100	100	0	80

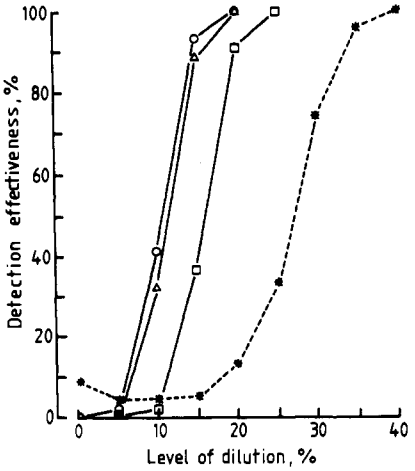

Figure 3. Effectiveness in detecting dilutions when using different subpopulations as reference sets: ○, White oranges, first season; △, Navel oranges, first season; □, White oranges, second season; discontinuous line, all juices (Figure 1).

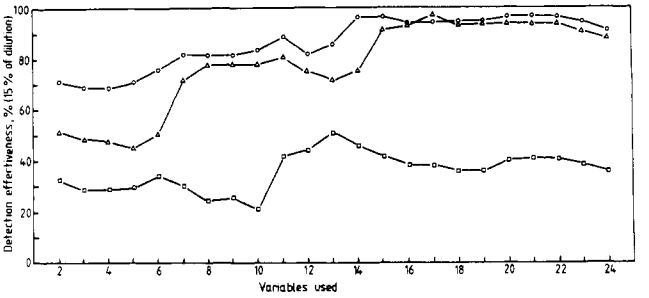

Figure 4. Effectiveness of each subpopulation in detecting 15% of dilution as a function of the variables used in computations (the variables listed in Table VI were used in the corresponding step): ○, White oranges, first season; △, Navel oranges, first season; □, White oranges, second season.

values and a covariance matrix from each group were used in each case, and hypothetically diluted juices were tested against their corresponding group. Results obtained using the whole set (Figure 1) are also represented in Figure 3 as a discontinuous line for comparative purposes. Dilution levels of, for example, 15% were detected in 94, 84, and 36% of the cases (respectively, in each subpopulation), whereas only 5% of detection was possible with the whole population. Moreover, no erroneous rejection of pure samples was observed in any case.

With each subpopulation to test the purity of samples from other subpopulations, the results were very different: as Table V shows, almost all pure juices were considered diluted.

The conclusion, previously stated (Brown and Cohen, 1983), is very clear: A suspicious juice must be tested exclusively against its own population if a true verdict is desired.

The analyst can have difficulties in knowing the population a juice came from, but these difficulties will decrease if juice labels show national origin, varietal source, and crop season.

Variable Selection. It can be observed from Figure 3 that detection effectiveness was much lower with data from White oranges of the second crop season. This is due to the higher variability of these data in relation to data from White and Navel oranges from the first season. As a consequence of higher variability, reflected on the covariance matrix, sample representation of White oranges from the second season spread (Figure 1) and dilutions were hardly detected. Uner these conditions variable selection may be interesting, thus removing variables of low discriminant power that increase χ^2 critical value without compensation in terms of detection effectiveness.

Table VI orders the variables in relation to their decreasing differentiation power between pure and diluted (15% of dilution) juices. The order was independently obtained for each subpopulation by stepwise discriminant analysis. Figure 4 shows the detection effectiveness in

Table VI. Stepwise Discriminant Analysis[a] between Pure and Diluted (15%) Juices, Order of Variables According to Their Decreasing Discriminant Power

step	subpopulation		
	White oragnes, first season	Navel oranges, first season	White oranges, second season
1	magnesium	magnesium	ash
2	glycine	ash	γ-aminobutyric acid
3	ash	valine	abs 280 nm
4	lysine	proline	methionine
5	isocitric acid	histidine	isocitric acid
6	proline	abs 443 nm	aspartic acid
7	γ-aminobutyric acid	lysine	potassium
8	glutamic acid	threonine	proline
9	aspartic acid	glycine	lysine
10	histidine	abs 325 nm	serine
11	abs 280 nm	asparagine	arginine
12	abs 325 nm	alanine	asparagine
13	threonine	calcium	glycine
14	serine	aspartic acid	alanine
15	potassium	glutamic acid	glutamine
16	glutamine	isocitric acid	magnesium
17	ornithine	potassium	abs 443 nm
18	alanine	glutamine	threonine
19	arginine	serine	valine
20	asparagine	abs 280 nm	histidine
21	calcium	ornithine	abs 325 nm
22	valine	methionine	ornithine
23	abs 443 nm	arginine	glutamic acid
24	methionine	γ-aminobutyric acid	calcium

[a] In each step the variables previously named were also used in computations.

relation to the number of variables used in the analysis, according to the arrangement on Table VI. For example, in the case of using four variables with the subpopulation constituted by White oranges from the first season, these variables were magnesium, glycine, ash, and lysine. As observed in Figure 4, detection effectiveness for White oranges from the second season reached a maximum using 13 variables. After this maximum, effectiveness decreased fast as the number of variables increased. With the other two subpopulations the effectiveness remained almost constant when 15 or more variables were used. It is difficult to conclude a general rule about the optimum number of variables from these results. Variable selection should be performed for each reference population and the optimum set of variables used in each case. This extra

work is not critical when considering present computation facilities.

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Registry No. K, 7440-09-7; Mg, 7439-95-4; Ca, 7440-70-2; aspartic acid, 56-84-8; glutamic acid, 56-86-0; asparagine, 70-47-3; glutamine, 56-85-9; serine, 56-45-1; threonine, 72-19-5; glycine, 56-40-6; alanine, 56-41-7; arginine, 74-79-3; γ-aminobutyric acid, 56-12-2; proline, 147-85-3; valine, 72-18-4; methionine, 63-68-3; ornithine, 70-26-8; lysine, 56-87-1; histidine, 71-00-1; isocitric acid, 320-77-4; citric acid, 77-92-9; sucrose, 57-50-1; glucose, 50-99-7; fructose, 57-48-7.

Supplementary Material Available: Raw data of White and Navel oranges (4 pages). Ordering information is given on any current masthead page.

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Characterization of the Kinetics of Breakdown of Protein Stabilized Oil in Water Emulsions

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The breakdown of oil in water emulsions, after initial formation, in the presence of 0.1% (w/v) whey, potato, pea, or soy proteins at pH 4–8 was monitored by measuring the absorbance at 500 nm of emulsions diluted in 0.1% (w/v) SDS after various time periods. Emulsion breakdown/absorbance decay was asymptotic, decreasing to an equilibrium value that was pH and protein dependent. Application of nonlinear modeling techniques revealed breakdown of protein-stabilized emulsions to follow first-order kinetics ($p < 0.001$). Use of a two-phase first-order model to characterize emulsion breakdown is also discussed. Results from this study emphasize the need to use appropriate statistical techniques to analyze replicated emulsion breakdown data; failure to do so could lead to biased estimates of kinetic parameters.

The ability of a protein to stabilize an oil in water emulsion is one of the most important functional properties with respect to application in food products such as finely comminuted meats, soups, cakes, and salad dressings. The dispersion of oil into water greatly increases the interfacial area and thus, also, the free energy of the interface between the two phases. As a result, a thermodynamically unfavorable environment is created, and this is reflected in an unstable emulsion. Due to their amphoteric nature and relatively large molecular weights, proteins are capable of adsorbing at the oil/water interface, thereby decreasing the interfacial tension between the two phases (Stainsby, 1986). The surface-active properties of proteins serve to lower the free energy of the oil/water interface and thus provide for a more thermodynamically stable system.

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