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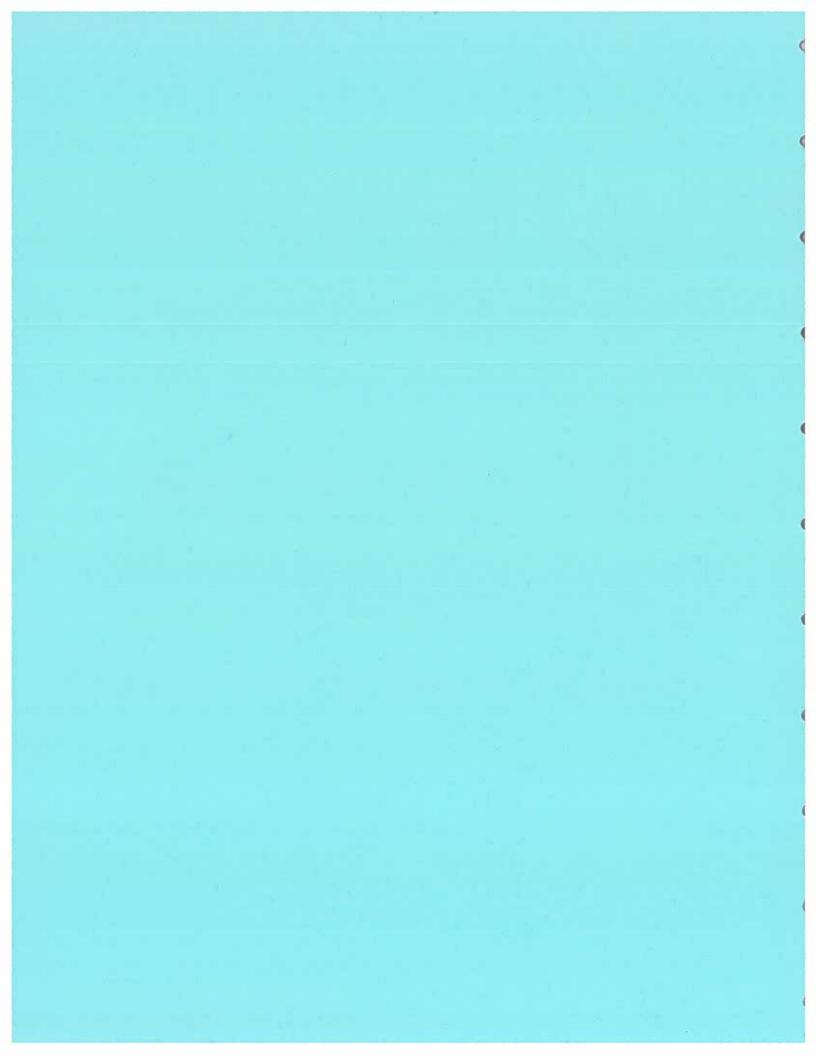
Effect of Washing on the Stability of Walleye Pollock Surimi During Frozen Storage

Kermit D. Reppond and Jerry K. Babbitt

February 1991

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EFFECT OF WASHING ON THE STABILITY OF WALLEYE POLLOCK SURIMI DURING FROZEN STORAGE

by

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ABSTRACT

The effect of insufficient washing on the functional properties of surimi was demonstrated by combining unwashed, refined mince with surimi. Addition of as little as 5% unwashed mince lowered punch force and gel strength, as well as torsion shear stress and strain. Expressible moisture and color were also affected by both the addition of mince and storage at -18°C. Incorporation of up to 15% unwashed mince produced surimi of lower, yet acceptable, quality. The decrease in functionality with time was greater for samples with more unwashed mince.

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INTRODUCTION

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An integral step in the preparation of surimi is the washing of minced fish flesh with water to remove components that would adversely affect its flavor, color, functionality, and stability during frozen storage (Kudo et al. 1973, and Lee 1984).

Traditionally, three batch-type washing cycles are used with a water-to-fish ratio of 3:1. At least one domestic processor is able to use a continuous in-line system of washing by mincing skinned fillets rather than headed and gutted or split fish (Babbitt and Reppond 1987). Whichever washing technique is used, determining the sufficiency of washing is based largely on subjective observation. Too much washing presents problems such as a loss of product because of the difficulty of dewatering the washed, minced flesh (Miyake et al. 1985). The object of this experiment was to investigate the effects of reduced washing on functionality by incorporating unwashed mince into surimi.

MATERIALS AND METHODS

Walleye pollock (Theragra chalcogramma) from 14 to 16 inches in length were processed at Alaska Pacific Seafood (APS), Kodiak, Alaska within 48 hours of capture. The fish were mechanically processed using Baader* 182 filleting and Baader 51 skinning machines. Skinless fillets were minced, washed, refined, and

^{*} Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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dewatered at APS using standard industrial practices (Lee 1986) and then transferred to our pilot plant. To obtain an unwashed but refined mince, fillets from APS were minced using a Baader 694 deboner. The unwashed mince was then refined using a Brown International Finisher equipped with screens with 0.094-inch (2.4 mm) holes. The paddles had 0.090 inch (2.3 mm) clearance from the screen and the finisher was run at a speed of 650 rpm. Samples of washed and unwashed mince were frozen and analyzed for moisture, protein, lipid, ash, and trimethylamine oxide (TMAO) content (Babbitt and Reppond 1987). Six 25 lb (11.4 kg) batches of processed fish mince were prepared containing 100% surimi, 95% surimi + 5% unwashed mince, 90% surimi + 10% unwashed mince, 85% surimi + 15% unwashed mince, 50% surimi + 50% unwashed mince, and 0% surimi + 100% unwashed mince. They are referred to as the 100, 95, 90, 85, 50, and 0% surimi samples. Each batch was then blended with 1 lb (454 g) sugar, 1 lb (454 g) sorbitol, and 0.075 lb (34 g) sodium tripolyphosphate for 10 minutes in a Leland Double Blade mixer. Five-pound subsamples of the mixtures were packed in polyethylene bags and frozen in a plate freezer at -40°C for 3 hours. The samples were then placed in master cartons and stored at -18°C until analyzed. The first samples were analyzed within 1 week and are referred to as having zero months of frozen storage.

Prior to analysis, samples were tempered overnight at 4°C and manually sliced into one-inch (2.54 cm) chunks. A portion of each sample reserved for determining moisture content. Then each

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surimi-mince mixture (2000 g) was mixed with NaCl (60 g) in a vacuum chopper (Babbitt and Reppond 1988). The resulting sol was cycled twice through a vacuum bagging machine to reduce air bubbles. Then, a mechanical stuffer equipped with a 0.5-inch (12.7 mm) diameter horn was used to stuff 50-mm flat width Saran casings for the punch test (Reppond et al. 1987) and 0.75-inch (19 mm) ID metal tubes for the torsion test (Kim et al. 1986). The gels were cooked for 40 minutes at 90°C, cooled in ice water, and held overnight at 4°C prior to testing.

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Determination of Hunter color and expressible moisture used gels prepared for the punch test. Hunter color was determined in triplicate on the cut surface of the gels using a Minolta Model Chroma II meter. Expressible moisture (EM) was determined using an Instron Model 1000. A 30-mm long sample was placed between sheets of Whatman No. 1 filter paper and subjected to 90% deformation at a crosshead speed of 50 mm/min. The Instron head was immediately elevated after the compression, and the weight gain in the paper was divided by the original weight of the gel to obtain the percent expressible moisture. Four replicates were measured for each sample. At each period of frozen storage, a one-way analysis of variance (ANOVA) test was performed to determine if the addition of unwashed mince to a surimi sample affected experimental results. A one-way ANOVA was also used to examine the effects of storage time on the surimi-mince mixtures. The least significant difference test was used to determine which sample means were different. Computations were performed using

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the Statistical Package for Social Science programming (Bowman and Cahill 1975). The level of statistical significance for all tests was set at $\alpha = 0.05$.

RESULTS and DISCUSSION

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Analysis of Uncooked Material

The moisture contents of the 100, 95, 90, 85, 50, and 0% surimi samples were 73.9, 74.7, 74.4, 74.3, 75.8 and 77.7%, respectively. Although the wide range in moisture content makes comparison of the 50 and 0% surimi samples with the other samples difficult, comparison among the samples with higher surimi content is possible since the range in moisture content was less than 1%. The much lower ash and trimethlyamine oxide content of the washed samples indicated that the washing was effective (Table 1). Hunter L*, a*, and b* values were higher in the 0% surimi sample than any of the 85-100% samples (Table 2).

Punch Test

Punch force decreased with addition of unwashed mince and longer storage time. At each time of frozen storage, the 100% surimi sample had the highest punch force (Table 3). Although statistically significant differences existed among the means of the 85-95% samples at 0, 3, and 12 months, the range in values was small and not meaningful as far as commercial quality is concerned. The 50% samples had lower punch force values than samples with less unwashed mince, and the 0% surimi (100% unwashed) sample had the lowest value of all. At 3 months, the

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gel from the 0% surimi was too weak to be tested by the punch method, and by 7 months the 50% surimi sample reached the lower limit of testing ability. Punch force values decreased with time for all samples, but not always smoothly. Between 0 and 3 months, the punch force values of the 95 and 85% surimi samples increased slightly, but significantly. Thereafter, punch force values decreased in these samples. Inhomogeneity of the sample could explain the increase, but it may simply reflect the degree of reproducibility of the gel preparation and punch test.

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Punch deflection was highest for the 0% surimi samples, probably as a result of their higher moisture content (Table 4). Although significant differences existed among the other samples at the first storage time, the differences were not great. At 3 months, small but significant differences existed among the 85-100% samples, while the 50% sample was much lower than these. At 7 months, the 100% samples had a higher mean value than the 85-95% samples. The 50% gel was so weak that it gave highly variable values, and hence comparison with other samples was not meaningful. At 12 months, the 95 and 100% samples had higher values than the 90% sample, and the punch deflection of the 85% sample was lower still. As with punch force values, punch deflection values tended to decrease with time, but not smoothly.

Changes in gel strength mostly followed the pattern of the changes in punch force (Table 5). The 100% surimi samples consistently had the highest gel strength. Differences among the

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85 to 95% samples were small until 12 months. Gel strength decreased with time, especially between 3 and 7 months.

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Torsion Test

The shear stress values of the 85 to 95% samples were initially somewhat higher than those of the 100% surimi sample (Table 6); however, the differences were probably not meaningful. At 3 months, the 85 and 100% samples had higher values than the 90 and 95% samples, while at 7 months the 85% sample had slightly higher values than the others. At 12 months, the 100% sample clearly had the highest values. The lack of a consistent trend at the end of each period of frozen storage may reflect the sensitivity of the torsion method to inhomogeneity of the samples or the inherent variability of the test itself. The 0 and 50% surimi samples had clearly lower shear stress values.

Torsion strain at failure was higher for the 100% sample than for the others at each storage period (Table 7). At 3 months, the effect of unwashed mince was evident as no two samples had the same value. At 7 months, the strain values of the 85-95% samples were higher than that of the 50% sample, and at 12 months the 95% sample had a higher strain value than the 90 and 85% samples. As with the other test results, strain decreased further with time, but in this case the decrease was consistent. The initial decrease in strain occurred between 3 and 7 months for the 100, 95 and 90% samples and between 0 and 3 months for the rest. Strain decreased between 7 and 12 months for the 85 to 95% samples but not for the 100% sample. Since

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strain decreased more quickly than stress, rigidity values increased with time in many cases making them useless so far as a test for quality was concerned. Therefore, rigidity values will not be discussed here.

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Expressible Moisture

Initially, no difference in expressible moisture (EM) values was noted except in the 0% surimi sample (Table 8). At 3 months, the mean EM of the 0% surimi was very high, indicating loss of functionality. Due to the large variance associated with the 0% surimi sample, the 50% sample was rated as not significantly different from the 85-100% samples. A one-way analysis of variance for the 3-month data excluding the 0% sample indicated that the 50% sample was significantly higher than the samples with more surimi. At 7 months, the EM of the 50% sample was very high, and the values for the 85 and 90% samples were slightly higher than the 95 and 100% samples. At 12 months, the 85% sample had the highest EM; the 90% sample was higher than the 95 or 100% samples. With the exception of the 95 and 100% samples, EM increased with time.

Hunter Color

Although analysis of variance indicated Hunter L* values varied with the addition of mince and with storage time, examination of the data revealed no consistent trend with either treatment (Table 9). The changes were probably due to normal variations in sample color and the high level of precision obtainable with the instrument used to determine Hunter color

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values. Hunter a* values, likewise, showed no clear trend although significant differences existed among each group of samples (Table 10). With Hunter b* values, addition of mince tended to increase values (increased yellow hue) at each period of frozen storage (Table 11). With the exception of the 100% surimi sample, Hunter b* values increased with time. Most changes occurred between 0 and 3 months and changed little, if at all, after that.

CONCLUSIONS

The importance of thorough washing in producing high-grade surimi was demonstrated as adding as little as 5% unwashed mince which lowered punch force, gel strength, and torsion shear stress even without extended frozen storage. Further incorporation of unwashed mince up to 15% did not cause additional decreases in these and other experimental results until subsequent frozen storage. Although the addition of 5 to 15% unwashed mince lowered functionality, the amount of the decrease was not so large as to make the mixtures unusable for commercial purposes. Torsion strain and expressible moisture were the most useful tests for detecting changes due to storage time or addition of mince, as the changes in these properties were more consistent than those in the other experimental results. Hunter b* values should be more useful in detecting insufficient washing than Hunter L or a values. Frozen storage did not seem to affect Hunter color of cooked gels in a consistent manner after 3 months

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at -18°C. The standard cryoprotectant mixture used in the production of surimi, and for this experiment was not effective in maintaining functionality in the material containing 50 or 100% unwashed mince.

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Table 1.--Initial composition of washed and unwashed walleye pollock mince.

	Washed	Unwashed
Moisture (%)	79.5	83.3
Protein (%)	20.0	17.0
Lipid (%)	0.4	0.4
Ash (%)	0.3	1.0
rmao† (mg N/100 g)	2.5	56.1

[†] Trimethylamine oxide.

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Table 2.--Hunter L*, a*, and b* of mixtures of surimi and unwashed mince (uncooked). The F-statistic and its associated probability (P) for a one-way analysis of variance is included.

Surimi, %	L*	a*	b*
100	49.9 ^{AB}	-0.9 ^B	-0.8 ⁸
	±1.3	±0.5	±0.6
95	47.7 ^{BC}	-0.6 ⁸	-1.18
	±1.8	±0.4	±0.6
90	45.8 ^c	-0.5 ⁸	~1.0 ⁸
	±2.8	±0.5	±0.4
85	48.0 ⁸⁰	-0.6 ⁸	-0.6 ⁸
	±2.6	±0.3	±1.3
50	49.9 ^{AB}	0.3 ^A	-0.5 ⁸
	±1.3	±0.1	±0.2
0 (52.8 ^A	0.3 ^A	1.0 ^A
	±2.7	±0.3	±0.3
-			
ANOVA F	3.59	6.00	4.46
P	0.032	0.005	0.016

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Means with a different letter were not significantly different (P < 0.05).

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Table 3.--Effect of adding unwashed mince on punch force (g) during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi		Storage time, months			
\$	0	3	7	12	F P
100	824 ^{Aa}	782 ^{Aa}	685 ^{Ab}	682 ^{Ab}	18.93
	±69	±50	±35	±49	0.000
95	630 ^{8Cb}	703 ^{8a}	603 ^{Bbc}	580 ^{8c}	20.74
,,,	±39	±27	±42	±39	0.000
90	665 ^{8a}	668 ^{Ca}	586 ^{8b}	534 ^{cc}	36.24
30	±40	±23	±40	±29	0.000
·· 85	606 ^{сь}	665 ^{ca}	591 ⁸⁶	504 ^{Cc}	42.49
00	±44	±24	±27	±30	0.000
50	422 ^{Da}	400 ^{Db}	239 ^{cc}	_t	203.1
30	±34	±11	±15		0.000
0	234 ^E	_	_	_	
v	±10				
NOVA					
F	227.0	234.3	270.0	42.56	
P	0.000	0.000	0.000	0.000	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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Table 4.--Effect of adding unwashed mince on punch deflection (mm) during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

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Surimi	Storage time, months				ANOVA
\$	0	3	7	12	F P
100	9.8 ^{Cb}	10.8 ^{Aa}	11.0 ^{Aa}	9.4 ^{Ab}	20.92
	±0.6	±0.5	±0.2	±0.7	0.000
95	10.1 ^{8Cb}	10.6 ^{ABa}	9.3 ^{8Cc}	9.1 ^{Ac}	34.42
	±0.5	±0.2	±0.3	±0.4	0.000
90	10.4 ^{Ba}	10.5 ^{8a}	8.9 ^{Cb}	8.5 ^{8c}	103.0
	±0.3	±0.3	±0.3	±0.4	0.000
85	9.6 ^{Cb}	10.3 ^{8a}	9.2 ^{8Cc}	7.6 ^{ca}	82.73
	±0.7	±0.3	±0.1	±0.2	0.000
50	9.9 ^{Ca}	8.0 ^{Cb}	10.0 ^{A8a}	_t	5.20
	±0.6	±0.2	±2.6		0.013
0	11.6^	-	-	_	
	±0.5				
NOVA					
F	16.39	127.2	5.38	33.76	
P	0.000	0.000	0.001	0.000	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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Table 5.--Effect of addition of unwashed mince on gel strength (g·cm) during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi		Storage time, months					
\$	0	3	7	12	F P		
100	807 ^{Aab}	847 ^{Aa}	755 ^{Ab}	643 ^{Ac}	12.99		
	±11	±72	±48	±64	0.000		
95	634 ^{8Cb}	745 ^{Ba}	560 ^{8c}	526 ^{8c}	57.40		
	±63	±38	±58	±52	0.000		
90	689 ^{8a}	661 ^{Ca}	519 ^{8b}	452 ^{cc}	78.86		
	±55	±39	±42	±38	0.000		
85	582 ^{Cb}	685 ^{ca}	543 ^{8b}	383 ^{Dc}	72.76		
	±79	±83	±30	±21	0.000		
50	418 ^{Da}	320 ^{Db}	242 ^{cc}	_†	25.85		
	±55	±13	±72		0.000		
0	271 ^E	-	-	-			
	±21						
AVOI							
	74.49	211.1	123.3	27.12			
f P	0.000	0.000	0.000	0.000			

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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Table 6.--Effect of adding unwashed mince on torsion shear stress (kilopascals) during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi	}		ANOVA F		
\$ *	0	3	7	12	P
100	75.3 ^{Ba}	76.2 ^{Aa}	66.0 ^{cb}	77.1 ^{Aa}	13.73
	±3.9	±3.9	±2.9	±2.1	0.000
95	85.3 ^{Aa}	68.9 ^{Cc}	73.2 ^{8b}	60.2 ^{8d}	94.89
	±3.3	±3.6	±1.4	±1.6	0.000
90	81.8 ^{Aa}	72.4 ^{Bb}	70.6 ^{8b}	57.0 ^{Bc}	61.93
	±2.4	±3.1	±2.7	±3.6	0.000
85	82.5 ^{As}	76.0 ^{Ab}	75.9 ^{Ab}	58.9 ^{8c}	50.63
	±5.5	±2.3	±2.9	±3.4	0.000
50	35.6 ^{Cb}	39.6 ^{Da}	32.7 ^{0b}	_t	11.72
	±2.8	±2.8	±2.4		0.000
0	36.2 ^{ca}	16.5 ^{Eb}		-	206.1
	±3.5	±1.6			0.000
IOVA					
F	241.5	522.6	392.1	55.77	
P	0.000	0.000	0.000	0.000	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).</p>

thyphen (-) indicates samples were too weak to be tested.

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Table 7.--Effect of adding unwashed mince on torsion shear strain during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi %	S		ANOVA F		
	0	3	7	12	P
100	2.61 ^{Aa}	2.53 ^{Aa}	2.01 ^{Ab}	2.03 ^{Ab}	72.79
	±0.11	±0.09	±0.05	±0.10	0.000
95	2.47 ^{ABa}	2.42Ba	1.93 ^{8b}	1.83 ^{8c}	147.5
	±0.10	±0.08	±0.03	±0.06	0.000
90	2.33 ^{Ba}	2.32 ^{ca}	1.87 ^{8b}	1.66 ^{cc}	96.73
	±0.07		±0.07	±0.05	0.000
85	2.40 ^{8a}	2.20 ^{0b}	1.93 ^{8c}	1.61 ^{cd}	249.4
	±0.06	±0.05	±0.06	±0.05	0.000
50	2.32 ^{8a}	1.75 ^{Eb}	1.11 ^{Cc}	_t	175.1
	±0.20				0.000
0	2.37 ^{8a}	1.30 ^{Fb}	_	_ #	206.1
	±0.17				0.000
OVA					
F	4.62	249.0	304.0	44.56	
P	0.003	0.000	0.000	0.000	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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Table 8.--Effect of addition on unwashed mince on expressible moisture (%) of surimi during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi	s	ANOVA F			
\$	0	3	7	12	P
100	0.99 ⁸	1.09 ⁸	1.44 ^{CD}	1.21 ^c	2.13
	±0.25	±0.02	±0.36	±0.33	0.146
95	1.15 ⁸	1.06 ⁸	1.15 ^D	1.41 ^c	2.65
	±0.33	±0.11	±0.09	±0.09	0.096
90	1.20 ⁸⁶	1.00 ^{8b}	1.67 ^{BCa}	1.90 ^{8a}	21.03
	±0.19	±0.19	±0.28	±0.07	0.000
85	1.26 ^{8c}	1.07 ^{Bc}	1.96 ^{8b}	3.01 ^{Aa}	28.13
	±0.43	±0.07	±0.24	±0.40	0.000
50	1.09 ^{Bc}	2.75 ⁸⁶	17.23 ^{Aa}	_t	2800
	±0.16	±0.19	±0.53		0.000
0	2.70 ^{Aa}	18.81 ^{Ab}	_	-	53.80
	±0.96	±4.28			0.000
OVA					
F P	241.6	66.40	1775	33.89	
P	0.000	0.000	0.000	0.000	

A-D Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

Hyphen (-) indicates samples were too weak to be tested.

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Table 9.--Effect of adding unwashed mince on Hunter L* values of cooked surimi gels during frozen storage at -18°C. The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi %	5	ANOVA F			
	0	3	7	12	P
100	76.7 ^{cc}	79.4 ^{Aa}	78.1 ^{8b}	78.7 ^{Aab}	20.93
	±0.7	±0.1	±0.5	±0.1	0.000
95	79.2 ^{A8a}	79.6 ^{Aa}	78.1 ^{8b}	79.1 ^{Aa}	9.63
	±0.3	±0.5	±0.4	±0.1	0.005
90	78.6 ^{ABc}	79.7 ^{Aa}	78.1 ^{8c}	79.0 ^{Ab}	21.76
	±0.4	±0.1	±0.2	±0.2	0.000
85	76.5 ^{cs}	79.4 ^{Aa}	79.1 ^{Aa}	78.0 ^{8ab}	7.36
	±1.5	±0.3	±0.2	±0.7	0.000
50	79.4 ^{Aa}	79.2 ^{Aa}	76.9 ^{cb}	_f	42.49
	±0.6	±0.2	±0.2		0.000
0	77.6 ^{8C}	78.3 ⁸	-	_	0.56
	±1.6	±0.1			0.50
OVA					
F P	4.93	7.68	18.48	9.72	
P	0.011	0.002	0.000	0.005	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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Table 10.--Effect of adding unwashed mince on Hunter a* values of cooked surimi gels during frozen storage at -18°C.

The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi	1	Storage tim	me, months		ANOVA F	
8	0	3	7	12	P	
100	-4.7 ^{Ec}	-3.3 ^{Db}	-2.8 ^{Aa}	-2.8 ^{Ba}	94.99	
	±0.2	±0.0	±0.2	±0.1	0.000	
95	-1.7 ^{Ca}	-2.8 ^{cc}	-3.5 ^{cd}	-2.4 ^{Ab}	91.45	
	±0.2	±0.0	±0.1	±0.2	0.000	
90	-1.8 ^{Ca}	-2.7 ^{BCc}	-3.2 ^{8d}	-2.2 ^{Ab}	135.2	
	±0.0	±0.0	±0.1	±0.2	0.000	
85	-2.9 ^{Db}	-4.2 ^{Ed}	-3.4 ^{BCc}	-2.2 ^{Aa}	175.7	
	±0.1	±0.1	±0.1	±0.2	0.000	
50	-1.3 ^{Ba}	-2.6 ^{8b}	-2.7 ^{Ab}	_1	108.5	
	±0.2	±0.1	±0.1		0.000	
0	1.1 ^{Aa}	-1.7 ^{Ab}	-	-	1722	
	±0.0	±0.1			0.000	
IOVA						
F P	648.5	318.0	22.66	25.08		
P	0.000	0.000	0.000	0.000		

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.



Table 11.--Effect of adding unwashed mince on Hunter b* values of cooked surimi gels during frozen storage at -18°C.

The F-statisic and its associated probability (P) from a series of one-way analysis of variance (ANOVA) tests are included.

Surimi	5		ANOVA F		
\$ *	0	3	7	12	P
100	1.0°	1.1 ^E	0.6 ^D	0.9 ^c	3.29
	±0.3	±0.1	±0.2	±0.2	0.079
95	-0.2 ^{Db}	1.1 ^{OEa}	1.2 ^{Ca}	1.2 ^{Ca}	56.23
	±0.2	±0.2	±0.1	±0.1	0.000
90	0.1 ^{Db}	1.4Da	1.4 ^{Ca}	1.5 ^{8a}	61.62
	±0.1	±0.0	±0.2	±0.2	0.000
85	1.1 ^{Cc}	2.8 ^{Ca}	2.1 ⁸⁶	2.2 ^{Ab}	38.03
	±0.1	±0.2	±0.2	±0.3	0.000
50	2.7 ^{8c}	4.0 ^{8b}	4.9 ^{Aa}	_t	51.02
	±0.3	±0.1	±0.1		0.000
0	4.1 ^{Ab}	7.2 ^{Aa}	_	_	679.7
•	±0.1	±0.1			0.000
NOVA					
	140.3	504.3	528.8	49.74	
F P	0.000	0.000	0.000	0.000	

Means within column with a common uppercase letter were not significantly different (P < 0.05).

Means within row with a common lowercase letter were not significantly different (P < 0.05).

[†] Hyphen (-) indicates samples were too weak to be tested.

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