A Multi-Functional Plant Protein for Skin and Hair Care

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Abstract

This article discusses a specially developed gluten-free, multi-functional, hydrolysed wheat protein which has independent claims data to support its efficacy as a free-radical scavenger, as an active which is substantive to, strengths and repairs damaged hair, as an ingredient which reduces surfactant irritancy in formulations, particularly those for babies and sensitive skin and as a foam booster.

Introduction

Plants synthesise all amino acids independently compared to human beings and animals who are only able to generate internally the so-called non-essential amino acids. The essential amino acids have to be taken orally or applied topically to hair or skin in the case of cosmetic products. An exceptionally high yield of essential amino acids can be obtained from wheat.

I Test Product description

The test product* is is a high purity wheat protein hydrolysate obtained by a gentle enzymic digestion process which produces a cosmetic raw material which has the following important characteristics:

- Water soluble
- Preservative-free
- Gluten-free
- 100% plant-derived
- Attractive functional properties for both skin and hair-care
- Appearance: light, white odourless powder
- Molecular weight distribution: The average molecular weight is ca. 2200 Daltons, which represents an ideal value for both dual moisturising and film-forming properties.

Functional Properties	Technical Properties
• Free radical scavenger	• Thickens and stabilises foam in detergent formulations
• Moisture-retaining and balancing properties	• Facilitates buffering of cosmetic formulations
• Hair conditioning and repairing effects	• Stabilises emulsions
• Promotes curl in hair and eye lashes	• Improves adhesion and durability of face powders
• Improves tolerance of skin and eyes to surfactants	• Film-forming activity

Table 1 Functional and technical properties of the test product

Below is the typical amino acid composition (g/100 g) of the test product*								
ca.	2.3 %	Alanine	ca.	2.2 %	Histidine	ca.	12.3 %	Proline
ca.	2.8 %	Arginine	ca.	3.8 %	Isoleucine	ca.	4.8 %	Serine
ca.	3.0 %	Aspartic Acid	ca.	6.9 %	Leucine	ca.	3.2 %	Threonine
ca.	2.0 %	Cystine	ca.	1.2 %	Lysine	ca.	0.8 %	Tryptophan
ca.	37.2 %	Glutamic Acid	ca.	1.5 %	Methionine	ca.	3.5 %	Tyrosine
ca.	3.1 %	Glycine	ca.	5.2 %	Phenylalanine	ca.	4.2 %	Valine

The following groups of amino acids are present:

- Essential amino acids: cystine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine.
- Semi-essential amino acids: arginine and histidine.
- Cystine and tyrosine may be partially synthesised organically from methionine and phenylalanine.
- The content of essential and semi-essential amino acids in the test product* is as high as 49.6% of the protein content.

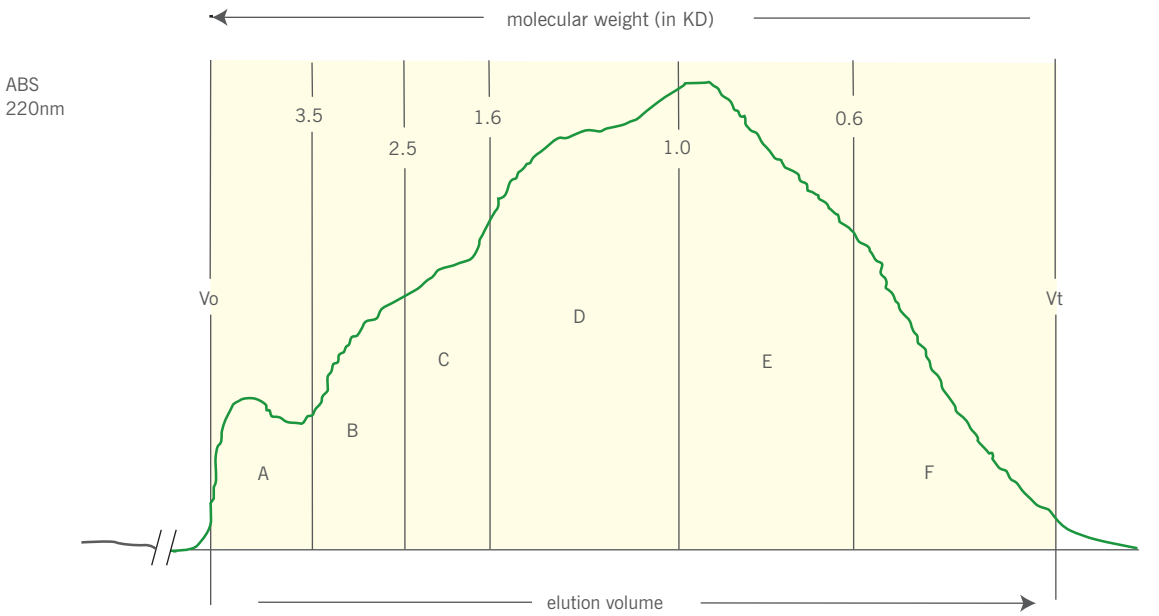
Gluten-free

- There have been recent consumer concerns that cosmetic products containing gluten could cause problems for people with gluten-intolerance, especially if these come in contact with the nose or mouth. For this reason the test product* has been tested by an external laboratory for the presence of gluten using an ELISA method.
- < 20 mg/kg gluten were found.

This conforms with the legislation in the food industry in the EU, Switzerland and USA which stipulates that "gluten-free" products must contain < 20 mg/kg gluten.

Molecular Weight Distribution

The average molecular weight of the test product is ca. 2200 Daltons, which represents a well balanced value for dual moisturising and film-forming properties. The pattern of molecular weight distribution is shown below (Gel Filtration Chromatography):



The relative area percentages of fractions A to F are: 5.3, 9.7, 9.5, 30.6, 30.4 and 14.5% (±10% rel.).

Gel Filtration Chromatography. Column: TSK HW-40 500x10mm.
Eluant: 0.1M NaCl, 0.1M phosphate buffer pH 6.8.
Flow: 0.4 ml/min. Detection: UV 220nm.

II Efficacy tests

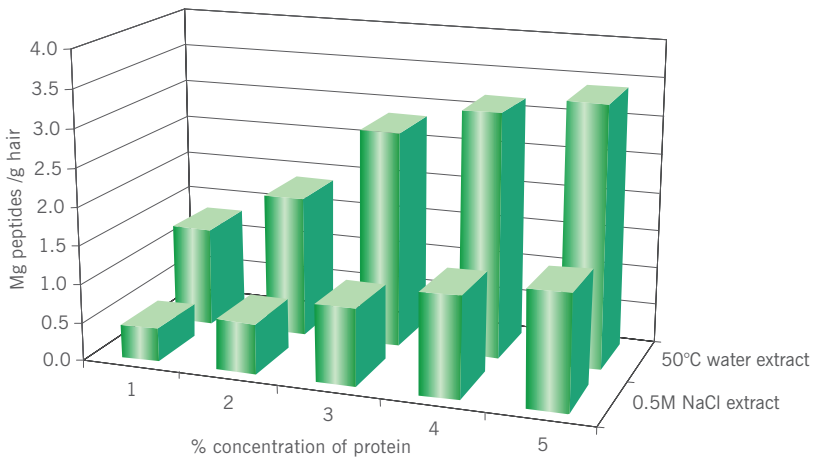
IIa Hair substantivity

Method

Adsorption to and removal from the hair shaft of peptides was measured by a quantitative analysis of amino-terminal groups using a fluorescamine reaction and fluorometric determination[10].

Results

- The adsorption of the test product peptides on both normal (Figure 2) and damaged (Figure 1) hair has been shown to occur even under short exposure times (30°C for 15 minutes) from a simple aqueous solution.
- An increasing adsorption of peptides is recorded up to a 5% protein concentration.
- Both small and large peptides are bound to the hair, but higher molecular weight peptides can be easily removed.



Wheat (Gluten-free) Herbaprotein Substantivity on damaged hair

Figure 1 The amount of Wheat Herbaprotein™ absorbed/desorbed on damaged hair

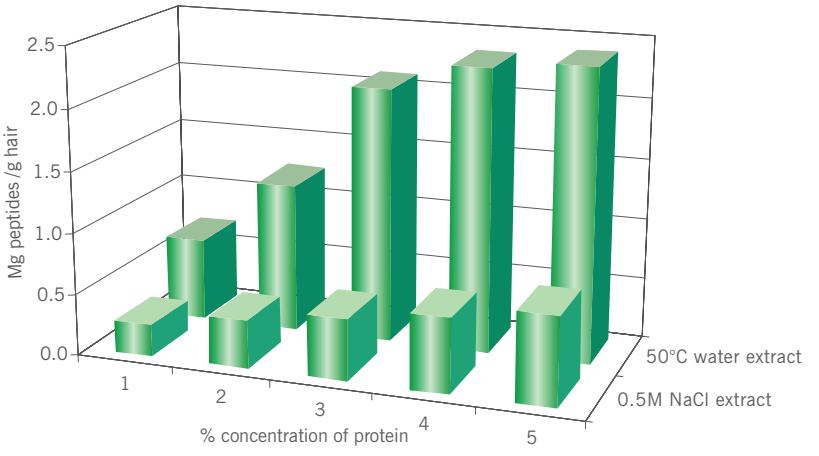


Figure 2 The amount of Wheat Herbaprotein™ absorbed /desorbed on virgin hair

IIb Free radical scavenging activity

Method

The test product* was evaluated for free radical activity and compared with other vegetable protein hydrolysates and a wheat protein hydrolysate with different molecular characteristics. The method used is based on the determination of the decrease in viscosity of hyaluronic acid solutions due to the effect of free radical activity and of its inhibition in the presence of free radical scavengers[11]. Hydroxyl radicals are generated by the action of xanthine oxidase on xanthine and these depolymerise the hyaluronic acid thereby producing a decrease in viscosity. This decrease of viscosity is prevented in the presence of free radical scavengers.

For each of the samples, mixtures shown in Table 2 were tested.

How the results are expressed:

- The measured viscosity in Control 1 (Va) is considered to be 100%.
  - (VA = 100% = Va%).
- The % viscosity of Control 2 = Vb x 100/Va = Vb%.
- The % viscosity of Test sample = Vc x 100/Va = Vc%.
- The free radical activity RA = Va% - Vb%.
- The scavenger activity of the sample RS = Vc% -Vb%.
- The % scavenging activity of the sample (% inhibition of hydroxyl radical activity) RS% = 100 x RS/RA.

Results

- The presence of the test product* in the test solution reduces the loss of viscosity of the hyaluronic acid solution caused by free radicals produced by the action of the enzyme xanthine oxidase on the substrate xanthine. This activity is due to the presence of disulphide bonds and aromatic amino acids in the peptides.
- The test product* (VcH) was a more effective scavenger of free radicals than the other commercial wheat protein hydrolysate tested (VcC).

Control 1 (Va)	Control 2 (Vb)	Test sample (Vc)
Hyaluronic acid	Hyaluronic acid	Hyaluronic acid
Phosphate buffer	Phosphate buffer	Phosphate buffer
Xanthine oxidase	Xanthine oxidase	Xanthine oxidase
Xanthine	Hydrolysate	Xanthine
		Hydrolysate

Table 2 Test solutions

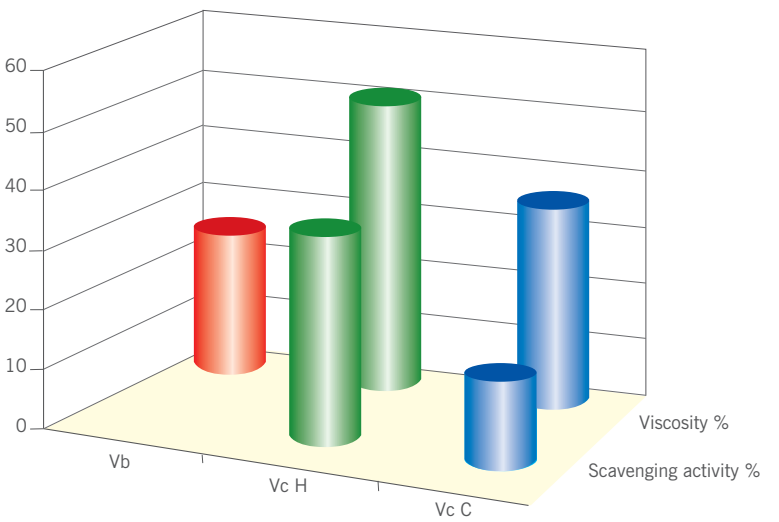


Figure 3 % viscosity and % free radical scavenging activity

Test sample	Viscosity % VC%	Scavenging activity % RS%
Vb Blank without xanthine	24.8	0
VcH Wheat Herbaprotein™· Average MWt 2,200 D. Isoelectic point 5.2	50.9	34.7
VcC Other Commercial wheat protein hydrolysate. Average MWt 2,000 D. Isoelectic point 5.0	34.8	13.3

Table 3 % Viscosity and % free radical scavenging activity

IIc Reduction of surfactant irritancy

Introduction

Frequent and cumulative exposure of the skin to surfactants based on anionic surfactants can result in a clinically and instrumentally identifiable skin damage. Excessive removal of the protective hydrolipidic layer and interaction with the proteins of the stratum corneum cause loss of the physiological barrier function and can give rise to multiple adverse effects like dryness, erythema and roughness. The denaturing action of anionic surfactants upon epidermal keratin is based on one or a combination of the following mechanisms: the hydrocarbon tail of surfactants penetrates into apolar regions of the keratin, replacing the conformation-stabilising hydrophobic interactions by ligand-segment interactions[1]. The ionic head produces attraction-repulsion forces on the charged groups of the keratin disrupting its structure[1]. The formation of excess of positive and negative charges causes additional osmotic pressure with consequent swelling of the matrix and increase of permeability[2]. The protection of epidermal proteins from the denaturing action of anionic surfactants and the improvement of their skin tolerance is normally carried out by the addition of surface actives able to lower the CMC, forming larger, less penetrating micelles[3,4].

Protein hydrolysates are also reported as effective additives. Claimed mechanisms of their action are:

1. Proteins form complexes with surfactants by means of ionic, hydrophobic and hydrogen bonds: this reduces the concentration of the free monomeric species of the anionic, those able to penetrate membranes[5].
2. Exogenous proteins interact with the skin keratin by means of weak but numerous bonds forming a protective colloidal layer which undergoes the denaturing attack of tensides[6].

This study focused on quantification and comparison of the protective effect of mild surfactants and a wheat protein hydrolysate (test product*) in simple and complex tenside systems based on anionic detergents.

Method

Two different trial designs were adopted in the present study in order to assess the efficacy of mildening additives in distinct exposure conditions and to distinguish between the different possible mechanisms of their protective effect, related to the duration and characteristics of the topical application[9].

Test Materials[9]

- Anionic surfactants: Sodium Lauryl Sulphate (SLS), Sodium Laureth Sulphate (LES), Sodium Linear C_{14-16} Olefin Sulphate (OS)
- Amphoteric and non-ionic surfactants: Cocoamidopropyl Betaine (BET), Cocamidopropylamine Oxide (MAO), Alkyl C_{8-16} Polyglucose (APG), Cocoamide DEA(CDEA)
- Test wheat protein hydrolysate*: average molecular weight 2200 Daltons

Subjects[9]

- Subjects were a group of healthy Caucasian volunteers, ages 19-60 years and free from skin diseases.

Exposure Models[9]

- **Single occlusion patch test** (8hrs). Although many researchers believe that the occlusion patch test can be misleading as a prediction of effects in a normal use situation[7], it has proved useful in comparative assessments of irritancy. Test solutions (50 ml) were applied on volar aspect of forearm using large (12mm) Finn chambers[9].
- **Washing test** (4 week controlled twice daily washing of forearm and inner forearm)[9]. This standardised washing test offers a more relevant and reliable assessment of the effect of products on the skin, than single and extended occlusion procedures[8].

Evaluation Models

- **Transepidermal Water Loss** (TEWL). The measurements were made on the forearm of subjects using a Tewameter™ and following procedure described in ref. 9. The results are expressed as:

TEWL relative % increase = $((X_t/X_c)_n - X_t/X_c)_b$ increase) X 100

Where:

$X_t/X_c)_n$ = ratio of the values between treated and control sites at time n

$X_t/X_c)_b$ = ratio of the baseline values between treated and control sites

In order to refer to the TEWL increase of the solutions containing the anionic tenside and additive(s) to that of the pure anionic the following expression has been adopted:

TEWL normalised relative % increase = A X 100/B

Where :

A = TEWL relative % increase of solutions containing anionic and additives[9]

B= TEWL relative % increase of pure anionic solution

- Electrical Capacitance (Corneometer) The capacitance measurements were made using a corneometer and following the procedure described in ref. 9. results are expressed as:

EC relative % change = $((Y_t/Y_c)_n - (Y_t/Y_c)_b)$ X 100

Where:

$Y_t/Y_c)_n$ = ratio of the values between treated and control sites at time n

$Y_t/Y_c)_b$ = ratio of the baseline values between treated and control sites

Results

Single Occlusion Patch Test

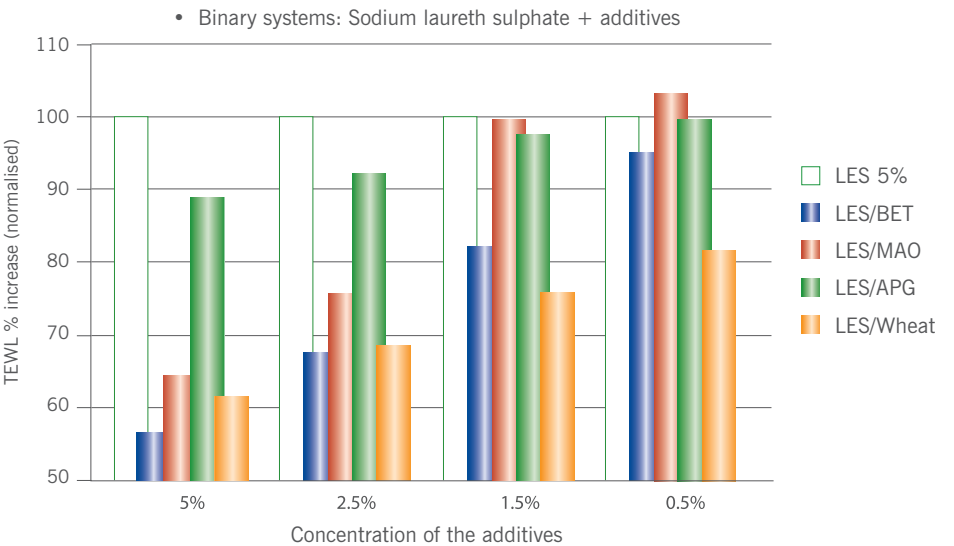


Figure 4 Patch test: TEWL rel. % increase (normalised) values for LES 5%+ additives at different concentrations

Sample	Concentration of the additive				
	5.00%	2.50%	1.25%	0.50%	0.00%
LES 5%					89.0 ± 20.7^{e}
LES 5% - BET	49.8 ± 9.3^{a}	59.9 ± 10.9^{b}	73.9 ± 13.4^{c}	84.1 ± 13.2^{e}	
LES 5% - MAO	56.6 ± 12.1^{b}	67.5 ± 11.2^{c}	88.4 ± 11.0^{e}	92.0 ± 12.9^{e}	
LES 5% - APG	78.9 ± 13.3^{d}	82.8 ± 14.5^{d}	86.7 ± 14.6^{e}	88.2± 13.0^{e}	
LES 5% - Wheat	55.2 ± 8.3^{b}	60.3 ± 8.4^{b}	67.7± 13.2^{c}	73.2 ± 15.1^{c}	

Table 4 Statistical data for Figure 4 TEWL rel. % increase mean values (n=20)±SD. 2-way (composition x conc.)
ANOVA: Fcomp=36; Fconc=40; Finter=5.3
Means with the same letter superscripts are not significantly different at the 95% confidence level. (Duncan Multiple Range Test).

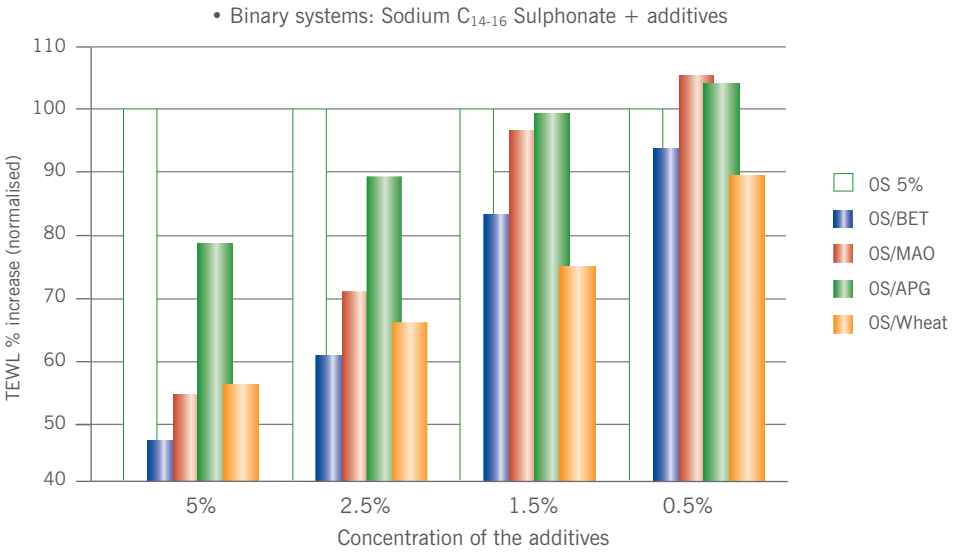


Figure 5 Patch Test: TEWL rel. % increase (normalised) values for Sodium C_{14-16} Sulphonate 5%+ additives at different concentrations

Sample	Concentration of the additive				
	5.00%	2.50%	1.25%	0.50%	0.00%
OS 5%					136.0 ± 28.6^{e}
OS 5% - BET	62.6 ± 10.5^{a}	82.1 ± 12.6^{b}	112.2 ± 17.4^{d}	125.9 ± 24.2^{d}	
OS 5% - MAO	72.2 ± 13.0^{b}	95.5 ± 16.5^{c}	129.9 ± 22.8^{e}	141.9 ± 19.6^{e}	
OS 5% - APG	104.8 ± 17.8^{c}	119.2 ± 21.0^{d}	131.9 ± 21.6^{e}	139.7 ± 24.3^{e}	
OS 5% - Wheat	74.9 ± 12.1^{b}	89.5 ± 18.9^{b}	99.9 ± 14.2^{c}	118.7 ± 20.7^{d}	

Table 5 Statistical data for Figure 5 TEWL rel. % increase mean values (n=20)±SD. 2-way (composition x conc.)
ANOVA: Fcomp=33; Fconc=45; Finter=6.4 Means with the same letter superscripts are not significantly different at the 95% confidence level. (Duncan Multiple Range Test).

• Multi-component systems: Sodium laureth sulphate + additives

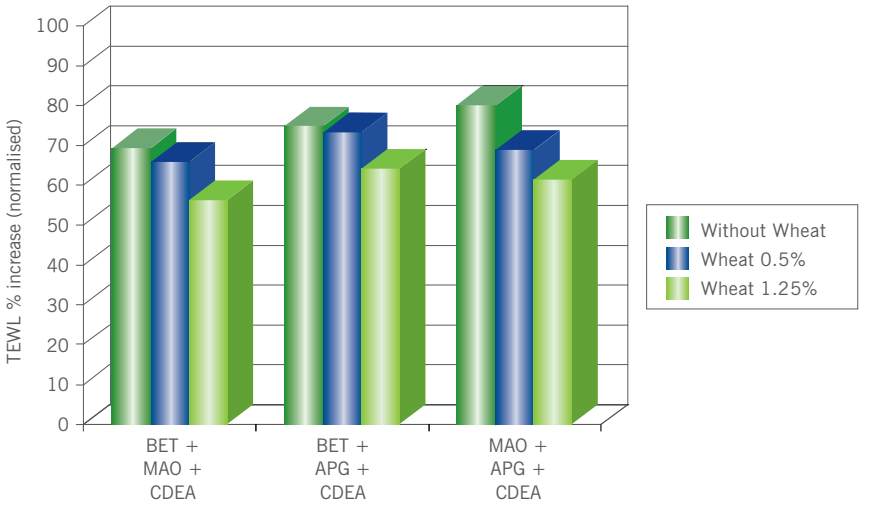


Figure 6 Patch Test: TEWL rel. % increase (normalised) values for LES 5%+ additives at different concentrations

Sample	Concentration of Wheat (Gluten-free) Herbaprotein			
	1.25%	0.50%	0.00%	Without additives
LES 5%				89.0 ± 20.7^{d}
LES 5% - CDEA - BET+MAO	50.9 ± 6.5^{a}	59.8 ± 11.0^{b}	62.2 ± 12.1^{b}	
LES 5% - CDEA - BET+APG	57.0 ± 8.7^{b}	65.8 ± 12.4^{c}	66.7 ± 9.5^{c}	
LES 5% - CDEA - MAO+APG	55.3 ± 8.8^{b}	61.5 ± 9.1^{b}	71.2 ± 9.7^{c}	

Table 6 Statistics for Fig. 6 TEWL rel. % increase mean values (n=20)±SD. 2-way (composition x conc.)

ANOVA: Fcomp=46; Fconc=58; Finter=6.1 Means with the same letter superscripts are not significantly different at the 95% confidence level. (Duncan Multiple Range Test).

Forearm Washing Test

• Binary systems. Sodium Lauryl Sulphate + additives

The results of TEWL measurements (as TEWL rel. % increase and TEWL % normalised increase) are reported in the Figures 7 and 8 together with the statistical Table 7/8.

See Figures 7&8 and Tables 7/8 overleaf.

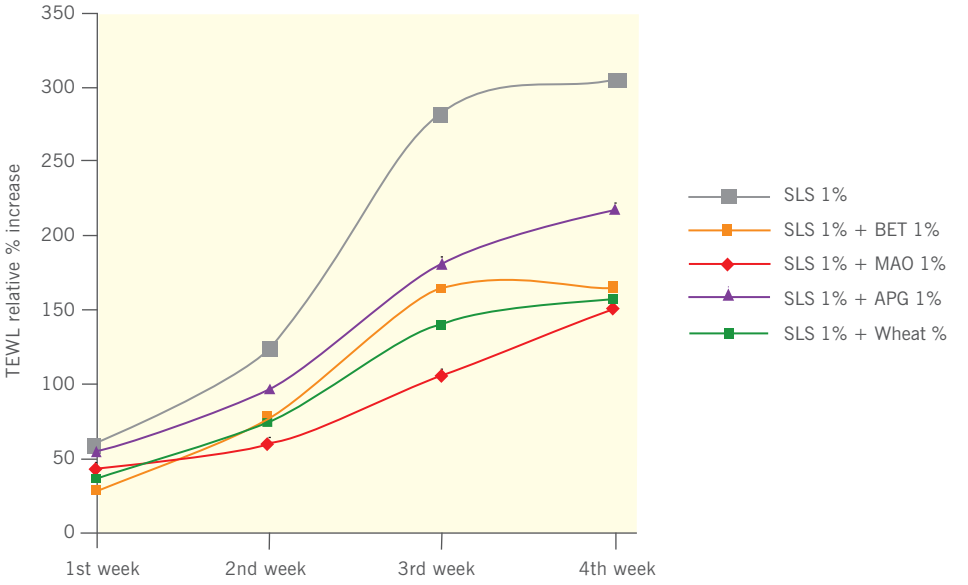


Figure 7 Forearm washing test: Sodium lauryl sulphate + additives (1%) - TEWL rel. % increase

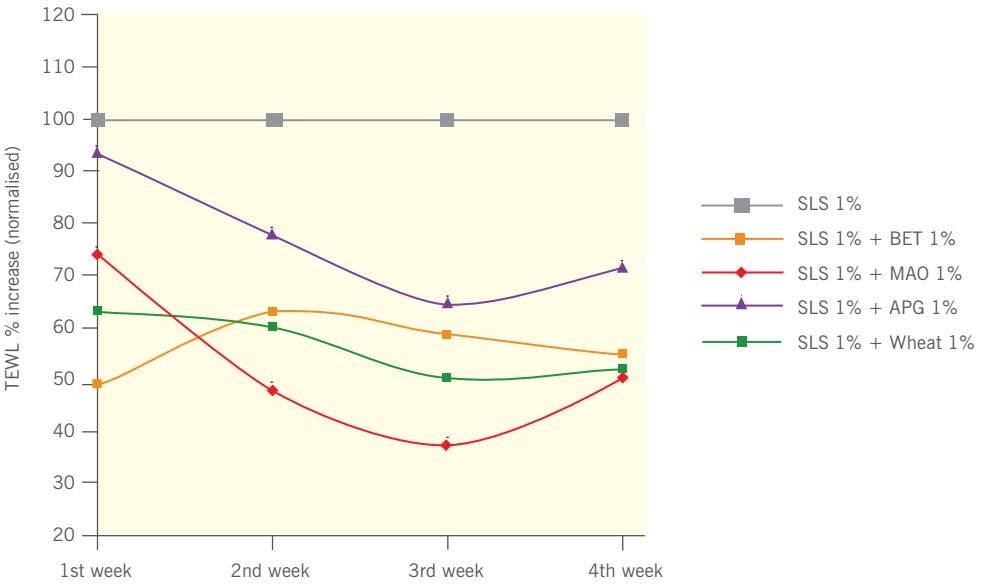


Figure 8 Forearm washing test – Sodium lauryl sulphate + additives (1%) - TEWL % increase (normalised)

Sample	Evaluation Time			
	1st Week	2nd Week	3rd Week	4th Week
SLS 1%	58.5 ± 14.4[b]	124.3 ± 31.1[e]	281.0 ± 54.3[j]	303.7 ± 77.7[k]
SLS 1% + BET 1%	28.5 ± 8.5[a]	77.9 ± 21.8[c]	164.8 ± 34.9[g]	166.0 ± 48.8[g]
SLS 1% + MAO 1%	43.4 ± 12.2[a]	59.7 ± 17.4[c]	105.7 ± 27.7[d]	152.3 ± 33.6[g]
SLS 1% + APG 1%	54.8 ± 13.7[b]	96.9 ± 24.9[d]	181.6 ± 44.0[h]	217.8 ± 39.7[i]
SLS 1% + Wheat 1%	36.7 ± 10.1[a]	74.7 ± 19.0[c]	140.3 ± 25.7[f]	158.1 ± 24.8[g]

Table 7/8 Statistical data for Figures 7 & 8 TEWL relative % increase mean values (n=12)±SD.

Friedman 2-way (composition x time) ANOVA: Fcomp=54; Ftime=111; Finter=9.5
Means with the same letter superscripts are not significantly different at the 95% confidence level.
(Duncan Multiple Range Test).

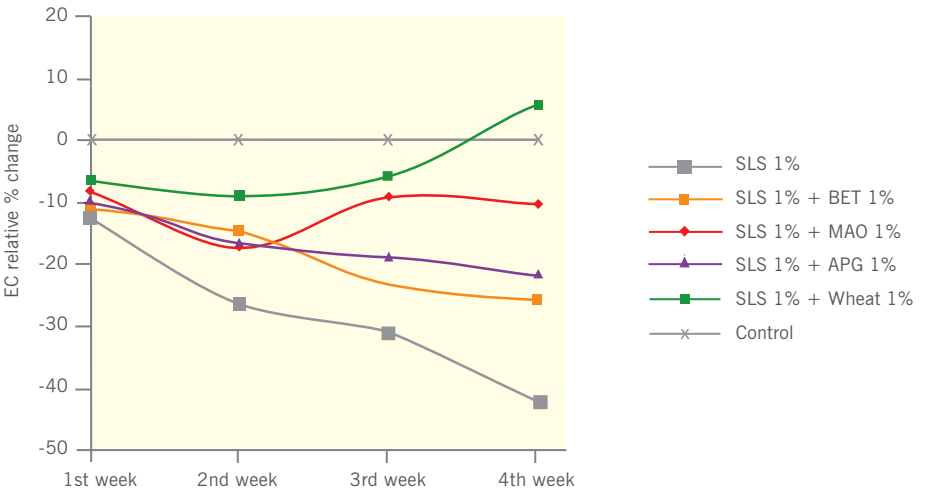


Figure 9 Forearm washing test – sodium lauryl sulphate + additives 1% Skin Capacitance Rel. % change

Sample	Evaluation time			
	1st Week	2nd Week	3rd Week	4th Week
SLS 1%	*	**	***	***
SLS 1%+BET 1%	*	*	**	**
SLS 1 + MAO 1%		*		
SLS 1%+APG 1%		*	**	**
SLS 1%+ Wheat 1%				

Table 9 Statistical data for Figure 8 EC relative % change mean course (n=12)±SD.

Friedman 2-way (composition x time) ANOVA: Fcomp=39; Ftime=3.6; Finter=2.7
Asterisks indicate differences versus control site (Dunnet Test)
*** $p < 0.001$
** $p < 0.01$
* $p < 0.05$

Conclusions on reduction of surfactant irritancy

- The different exposure models show analogous results in comparing and quantifying the protective potential of protein hydrolysates and mild tensides against the adverse changes occurring in the skin by intense topical application of anionic surfactants.
- The efficacy of the test product* gives results comparable to mild tensides in the single application tests and reveals superior in the washing trial.
- From the variety of tests presented the formulator of can identify useful strategies for appropriate blending of agents to increase the mildness of formulations for applications such as sensitive skin and baby products etc.
- In different models tested results suggest that in addition to the direct interaction with the surfactant molecule, the test product* is able to bind to the skin keratin, forming a continuous protective layer which shields the denaturing attack of anionics and helps preventing damage of the stratum corneum.

IId Foaming properties

Method

The Ross-Miles method (ASTM D1173-53) was used for the evaluation of the foam expansion and stabilisation properties of the test product*[12].

Results

- Small amounts of the test product (0.1-0.5%), improve the efficacy of detergents, increasing foam volume, stability and consistency and allowing a reduction of the amount of surfactants in formulations
- The foam expansion values were nearly 15 times higher than for commercial collagen hydrolysate

Not all protein hydrolysates are good foam enhancers, it depends on molecular size, net ionic charge, amino acid composition and sequence.

Conclusions from efficacy tests

Adsorption of the test product* peptides on both normal and damaged hair has been shown to occur even under short exposure times (30°C for 15 minutes) from a simple aqueous solution

- The test product* has been shown to be an effective free-radical scavenger
- The test product* increases the skin and eye tolerance of anionic surfactants
- Small amounts of the test product* (0.1-0.5%) improve the efficacy of detergents, increasing foam volume, stability and consistency and allowing the reduction of surfactants in formulations

III Cosmetic applications

The following cosmetic related activities of the test product* as shown below:

Plant proteins have excellent skin compatibility and deposit a protective film on the skin and hair. This film is both smoothing and moisturising.

Properties	Suggested cosmetic applications*
• Improves skin feeling and softness, smoothing • Moisturising, moisture-retaining • Free radical scavenger • Reduces surfactant irritancy, improves tolerance of skin and eyes • Substantive to hair, moisture-retaining, hair conditioning and repairing • Encourages curls (hair and eyelashes) • Film-forming • Thickness and stabilises foam: stabilises emulsions • Facilitates buffering of cosmetic formulations • Improves adhesion and durability of face powders	• Skin care especially anti-ageing, sensitive skins and babies and infant products • Sun care • Hair care, especially for damaged, brittle/dry hair • Bath and shower products, especially for sensitive skins and babies • Liquid and bar soaps • Detergents • Decorative cosmetics, especially face powders, foundation and mascara

Recommended level of use: 0.2 – 1.0 %.

Use in hair and skin care products.

Conclusion

The plant protein tested* was shown to be a highly interesting multi-functional active for a wide range of personal care applications with the following substantiated properties:

- Free radical scavenger /UVB protection
- Reduces irritancy of products due to other ingredients
- Substantive to and repairs damaged hair
- Improves quality and quantity of foam

In addition it is solvent-free, preservative-free and gluten-free.

* N.B. The gluten-free wheat protein used in this study is sold under our tradename of Wheat (Gluten-free) Herbaprotein™

References

1. Reynolds J.A., Herbert S., Polet H. & Steinhardt J. (1967) The binding of diverse detergent anions to bovine serum albumine. Biochemistry, 3: 937-47.
2. Zeidler U. (1988) Influence of surfactants on the swelling of epidermis. XV IFSCC Congress, London 1988.
3. Lang G. & Spengler J. (1986) Surfactants in cosmetic formulations: skin irritancy and physical properties. XIV IFSCC Congress, Barcelona, 1986.
4. Miyazawa K., Ogawa M. & Mitsui T. (1984) The physico-chemical properties and protein denaturation potential of surfactant mixtures. Int. J. Cosmet. Sci., 6: 33-46.
5. Pitt-Rivers R. & Ambesi-Impiombato F.S. (1968) The binding of sodium dodecyl sulphate to various proteins. Biochem. J., 109 : 825-30.
6. Berg A. & Lindner H. (1979) Schutz-Proteine in der Kosmetik. Parf. und Kosm., 60 : 74-8.
7. Finkey M.B. & Crowe D.M. (1988) The use of evaporimetry to evaluate soap induced irritation on the face. Bioeng. Skin, 4: 311-21.
8. Mills O.H., Swinywer L.J. & Kligman A.M. (1984) Assessment of irritancy of cleansers. XLIII Annual Meeting of American Academy of Dermatology 1984.
9. Wheat (Gluten-free) Herbaprotein™ : Surfactant irritancy reduction tests. Full test report.
10. Wheat (Gluten-free) Herbaprotein™ : Hair substantivity tests. Full test report.
11. Wheat (Gluten-free) Herbaprotein™ : Free radical scavenging tests. Full test report.
12. Wheat (Gluten-free) Herbaprotein™ : Foaming properties tests. Full test report.

Author Biography

Dr Jane Tiedtke, BSc and PhD in Microbiology spent 15 years with Rohm and Haas Company in France in both marketing and technical posts in their Consumer and Industrial Specialities Division. She joined Cosmetochem International Ltd. based in Switzerland in 2001 and currently holds the position of Head of Marketing.