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Assessment of chemical properties and biological activity of Thorne Research, Myco-Immune, and Immudyne nutritional quality NQ yeast ß-1,3 1,6-glucan

A sample of the commercially available liquid nutritional supplement from Thorne Research, Myco-Immune, and the Immudyne nutritional quality ß-glucan, NQ, were analyzed in parallel to compare relative similarity. Myco-Immune is a water soluble liquid extract of seven different mushroom fungal species whereas Immudyne NQ yeast ß-1,3 1,6-glucan is a water insoluble yeast cell wall particulate. As indicated below the proximate analyses of these samples are not comparable because of differences in purity and physical form.

Table I
Chemical composition of Immudyne NQ

Physical form and color to pass 60 mesh sieve	powder, white, odorless
Water and organic solvent insoluble highly purified powder	
Total aerobic plate count	<10 cfu/gram
Complement activation assay 1mg/ml serum (Bb peptide as µg/ml)	61
pH of 10% suspension in water	5 to 6
Proximate analyses	%
Total volatiles/moisture	6.03
Extractable fatty substances	not detectible
Non hydrolyzables (acid)	1.25
Ash	0.85
Protein by Kjeldahl nitrogen (6.25 x N%)	2.81
Total non-ß-glucan	11.06
Glucan*	88.94
Carbohydrate**	103.47
Glucose***	96.92
^{13}C-nmr ß-1,3 1,6-glucan	NMR-conforms to all carbon
	resonances and intensities for
	ß-1,3/1,6- D-Glucan
Limit of glucan detection by ^{13}C-nmr	>98.2
Glucose/Mannose compositional ratio	>90/1
Molecular weight distribution, gel permeation chromatography	
atomic mass units (amu),Daltons	
Weight average molecular weight, M_W	3,572,000
Number average molecular weight, M_N	29,300
Polydispersity, Weight average/Number average, M_W/M_N	122
Hydrodynamic volume molecular weight, Mz	15,063,000

* by difference from proximate analysis after correction of hydrolysis sample weight for volatiles, ash, protein, and non-hydrolyzable polymer

** ß-glucan phenol/sulfuric acid assay reducing sugar

*** after acid hydrolysis by specific enzyme reaction, coupled glucose oxidase-peroxidase

Thorne Research, Myco-Immune Liquid

This nutritional supplement comes as a dark brown liquid, Figure 1. The concentration was determined by vacuum oven drying as 16.6% solids, Figure 2. The solids are stated to be water solubles extractes from the edible mushrooms Cordyceps, Grifola, Ganoderma, Lentinula, Tremelia, Schizophyllum, and Trametes. The mushroom extracts in Myco-Immune have definite mushroom aromas.

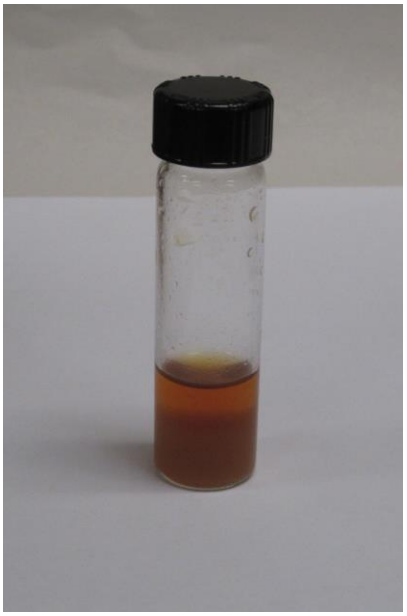

Figure 1. Myco-Immune liquid, as used 1 drop, 3 times per day

Figure 2. Myco-Immune solids from vacuum drying

The liquid Myco-Immune, 50 grams, was fractionated by dropping the liquid slowly into 90% ethyl alcohol and isolating first an alcohol insoluble, water soluble polymer, which was separated and dried to a powder: 1 gram, 11.2% of the 8.91 grams of solids in the 50 grams of liquid taken for analysis, Figure 3. This method isolates polysaccharides.

Figure 3. Myco-Immune insoluble polysaccharide from 90% ethanol precipitation

Figure 4. Myco-Immune fatty substance (dried), top layer in 90% ethanol

The alcohol soluble solute separated into an opaque fatty layer floating on top of the liquid, which was separated from the bulk of the alcohol and dried, Figure 4. The fatty alcohol layer weighed 1.25 grams, 14% of solids in the 50 grams of liquid. The remainder of the alcohol soluble material was isolated by drying and weighed 6.66 grams (74.7%) of dried syrup from the original 50 grams liquid, Figure 5.

Figure 5. Myco-Immune substance soluble in 90% ethanol (dried)

Figure 6. Immudyne NQ, white powder as analyzed

Each of these fractions give strong carbohydrate positive reactions with the phenol-sulfuric acid test as well as positive ninhydrin amino nitrogen reaction. Further compositional analyses of these fractions of Myco-Immune and Immudyne NQ are described below.

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Complement activation analyses comparing Immudyne NQ with Myco-Immune and its fractions

The complement activation capability of these glucans depends on the availability of the glucan chain to bind to the respective proteins in the secondary immune system pathway. The Immudyne NQ gives greater responses for protease activity at all three concentrations of glucan in serum compared to the Myco-Immune whole dried syrup or its fractions.

Serum (9 parts) was mixed with solution or suspension of the test substance (1 part). After incubation for 30 minutes at 37°C the mixtures were centrifuged to remove insoluble particles, and the supernatants tested for complement activation. Bb, the major fragment of Factor B, is a sensitive marker for alternative pathway activation. All samples were suspensions in normal saline (0.9% wt/volume).

Table II
Complement Activation Analyses

Sample tested	Concentration tested mg/ml suspension in serum, mg/ml	Weight peptide cleaved µg/ml serum
Immudyne NQ	1 0.4 0.1	57.47 46.03 23.47
Dried whole Myco-Immune sample	1 0.4 0.1	22.13 14.18 7.83
Polysaccharide powder from Myco-Immune liquid (11.2% of solids)	1 0.4 0.1	43.45 33.64 19.08
Myco-Immune 90% ethanol soluble solids dried syrup (74.7% of solids)	1 0.4 0.1	3.74* 3.17* 3.41*
Zymosan Positive control	5	54.08
Saline, 0.9% weight/volume Negative control		5.73

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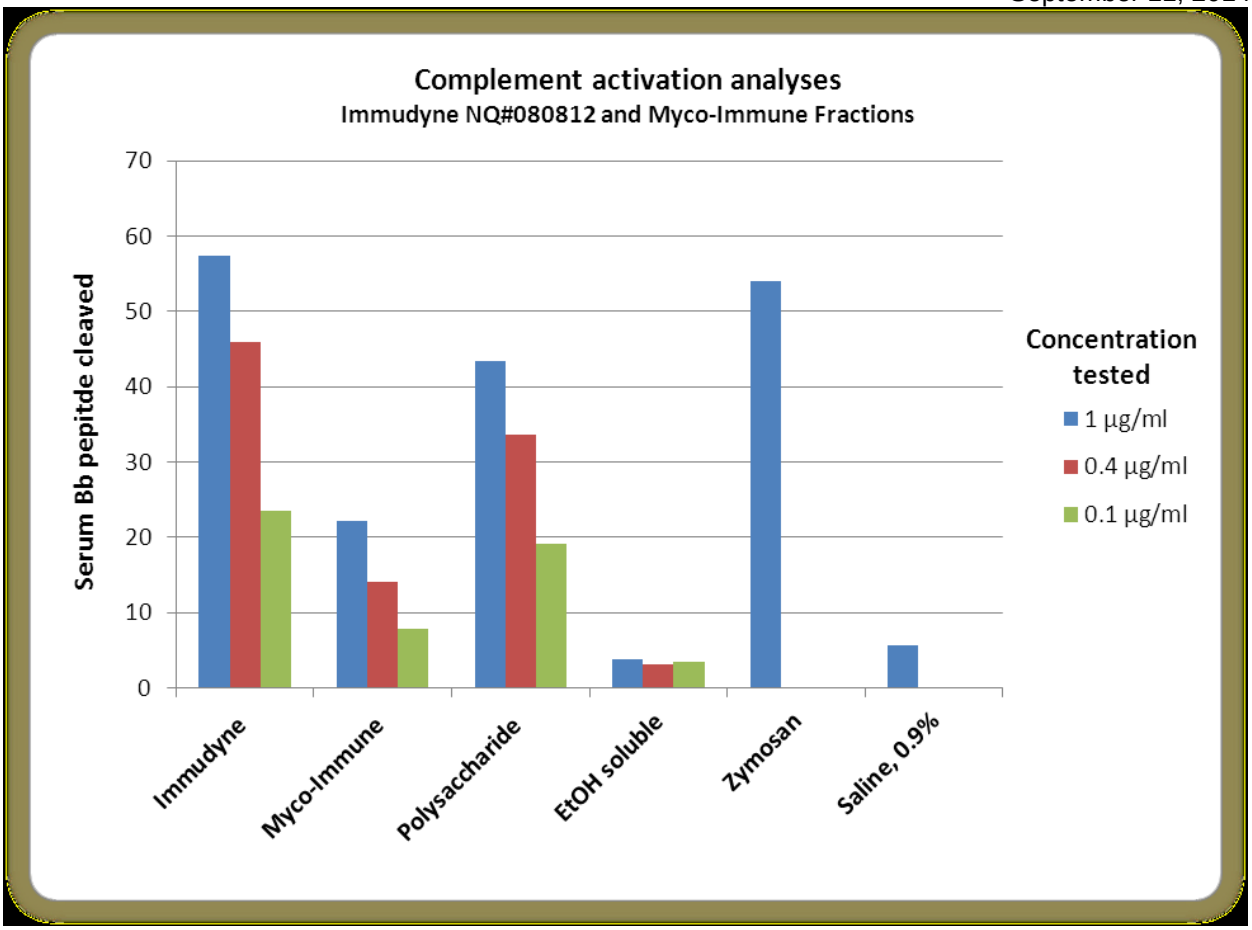

Spectrophotometric determination of polyphenolics and other browning reaction products in Myco-Immune and Immudyne NQ

The characteristic absorption of polyphenolics and other browning polymers at 278 nm in the ultraviolet spectrum is used to determine relative concentrations of these compounds in natural products. The Myco-Immune is very dark orange-brown in color which indicates a whole range of spectral colorants present. Such impurities inhibit binding of beta-glucans to specific sites on macrophages; careful removal of such compounds is done in the refining of the finished products. The differences in relative quantities of these substances in Myco-Immune liquid and its isolated fraction are compared to Immudyne NQ in the spectral overlay below. The water extraction of insoluble Immudyne NQ yields very little of these substances extracted into the water. The Immudyne NQ has been separated during processing from such browning products as well as polyphenolics. Immudyne NQ represents a substrate for immune activation that is quite pure.

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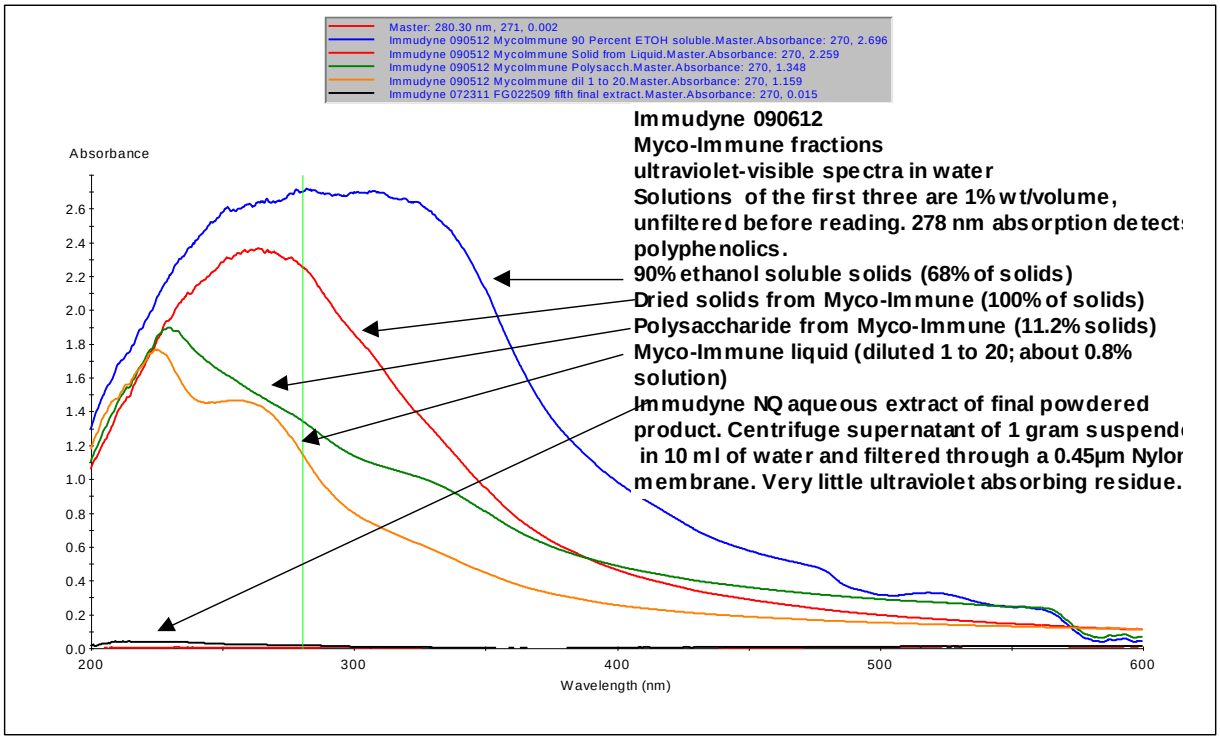

Carbohydrate composition of Myco-Immune and Immudyne NQ by hydrolysis and derivatization gas chromatography

The fractions described above from Myco-Immune and Immudyne NQ were hydrolyzed in acid and derivatized for separation of the sugar component by gas-chromatography. The sugar profiles for each Myco-Immune fraction as well the Immudyne NQ are shown in this table.

Table III

Sugar*	Immudyne NQ %	Myco-Immune polysaccharide %	Myco-Immune 90%EtOH soluble %	Myco-Immune dried (whole solids) %
fucose	None	0.37	0.28	0.57
arabinose	None	2.94	4.58	11.83
xylose	None	2.47	1.08	1.87
mannose	0.28	6.7	None	3.75
galactose	None	3.36	0.62	0.44
glucose	99.72	85.83	93.44	81.53

*The percentage is that for distribution of all the sugars present and not quantitatively representative of all the solids in the sample.

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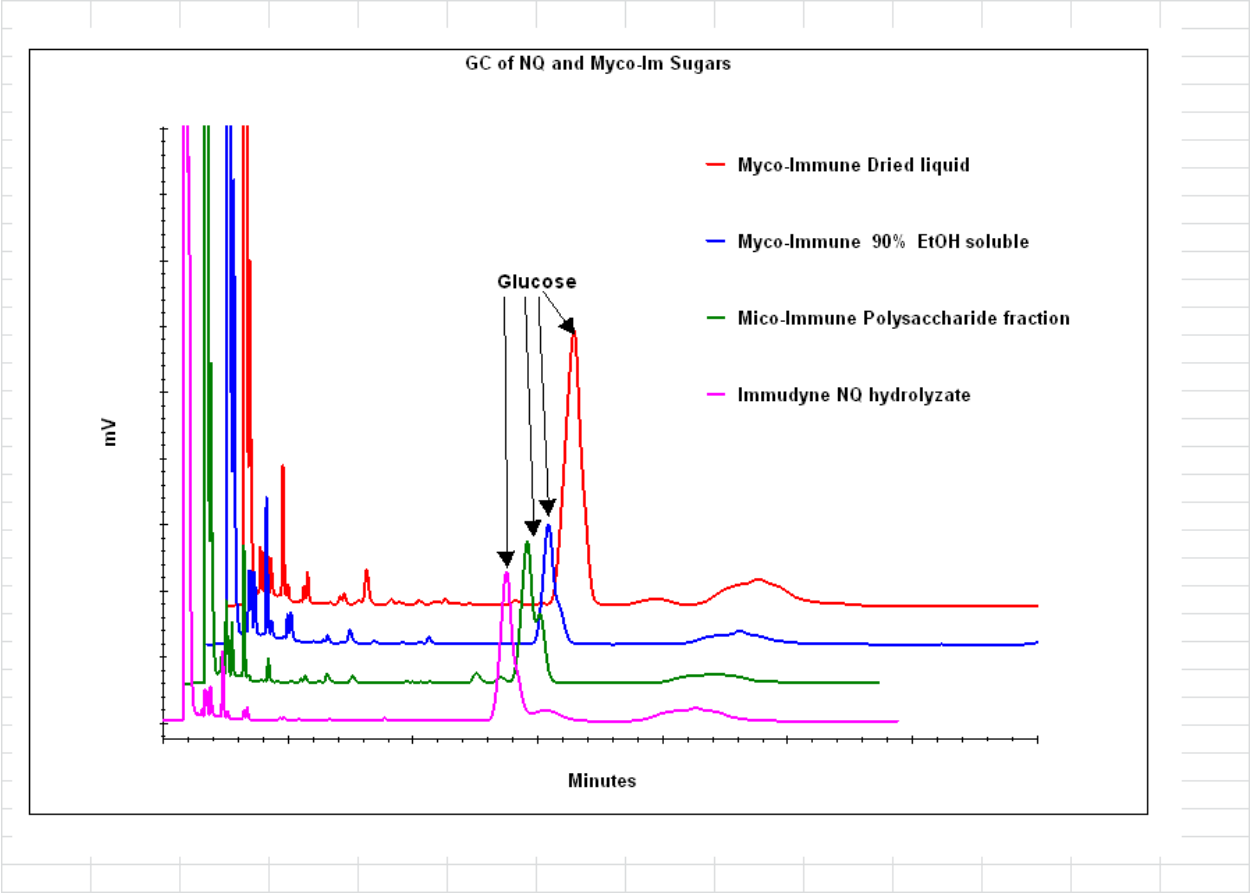

The Immudyne NQ is predominantly a glucan with only a trace 0.28% of mannose carried through from the yeast cell wall mannan originally present before extractions.

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Size exclusion liquid chromatography to determine relative molecular weights of components in aqueous solutions of the products

Unlike the previous table of individual sugars present this determination is carried out on the products and fractions in solution without previous breakdown by hydrolysis and derivatization. The separation of polymers from lower molecular weight materials in the water solutions gives an indication of the concentrations of most probable components to be biologically active. The Immudyne NQ is insoluble in water except for trace amounts of glucan oligomers hydratable from the original particulate. These show up as a small amount of high molecular oligomer that is not separated on the column but is excluded and appears as a peak. The exclusion limit of this column is about 2000 atomic mass units. The Myco-Immune fractions contain much ultraviolet absorbing material that separates in this procedure into many peaks, some of which are excluded and others of which are separated into many peaks. Identification of these ultraviolet absorbing peaks is difficult without mass spectrometry. However, in the ultraviolet detection these are certainly phenolic or electronically conjugated molecules in some high concentration. The mass detection by refractive index gives a reasonable quantitative distribution of the molecular types which are represented as polymers, oligomers, or simple sugars. The percentage of each peak represents the distribution of these molecules in the Myco-Immune and Immudyne NQ.

Table IV
Relative percentages of components in the Myco-Immune fractions

Retention Time	Component Name	Myco-Immune Dried solids %	Myco-Immudyne Polysaccharide %	Myco-Immune 90% EtOH soluble %
4.37	Polysaccharide	16.77	77.78	11.93
5.22	Unknown i	0.15	0.84	0.06
5.75	Unknown 2	0.32	0.91	0.15
6.43	Unknown 3	0.60	ND	0.21
7.23	Trisaccharide	6.43	ND	1.61
8.08	Unknown 4	0.17	ND	0.19
8.68	Disaccharide	11.69	4.66	13.07
9.23	Glucose	57.07	12.86	64.58
10.77	Unknown 5	0.16	0.68	0.18
11.23	Unknown 6	0.40	1.68	0.39
11.85	Unknown 7	0.30	0.59	0.07
12.53	Unknown 8	4.65	100	4.61
13.72	Unknown 9	0.26	ND	0.49
14.23	Unknown 10	0.30	ND	0.29
15.43	Unknown 11	0.35	ND	0.17

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Table V
Carbon-13 nuclear magnetic resonance spectra of the Immudyne NQ and Myco-Immune polysaccharide fraction

ASSIGNMENTS FOR CARBON-13
NUCLEAR MAGNETIC RESONANCE SPECTRA
September 7, 2012

	B-1,3 1,6-Glucan Immudyne NQ		Myco-Immune polysaccharide fraction	
Assignment	ppm	Intensity height (mm)	ppm	Intensity height (mm)
Aromatics from protein	None			
C-1, beta	103	112	105	35
C-1, beta	102	24	101	20
C-1 alpha	None		98.0	85
			93.0	55.0
C-3	86	115	None	
			77	100
C-5	76	7	None	
C-5	75	159	75	95
C-2	74	142	None	
	73	21	None	
	73	65	None	
			72	65
			71	100
C-6' (branched)	70	14	None	
C-4	68	128	None	
			62	105.0
C-6	61	157	61	25
			57	35
	39.6	DMSO, d-6, solvent.intensity. ca. 1000		
Lipid aliphatics - methylenes, olefins, and methyl groups	None		20	30
			19	60
B-1,3 1,6-glucan	Complete spectrum		Complete spectrum	
Soluble glucomannan	None			
Ratio of C-6 intensity/ C-6' intensity (Degree of branching)		11	None	
Ratio of C-3 intensity/ C-6' intensity (Degree of branching)		8	None	

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The Myco-Immune polysaccharide fraction described above was chosen for comparison of carbon-13 chemical shift data to discern similarities in the two products because the polysaccharide fraction exhibited the largest complement activation activity. The chemical shifts of the Myco-Immune polysaccharide do not have any correspondence with the Immudyne NQ spectrum. The composition of the dried Myco-Immune taken from the commercial liquid was estimated by size exclusion liquid chromatography to have 57% glucose and 16% polysaccharide. The isolated polysaccharide in the sample is 78% by weight of the powder but still has carried with it some glucose (13%). Other sugars besides glucose in the polysaccharide fraction determined by gas chromatography after hydrolysis are fucose, arabinose, xylose, mannose, and galactose in lower but significant amounts. The complexity of the carbon-13 n.m.r. for the Myco-Immune polysaccharide prohibits making any reasonable interpretation of the spectrum except to say that in the anomeric region of polysaccharide resonances there is about a 3 to 1 preponderance of alpha-D linked sugars to beta-D-linked sugars. The n.m.r. data for the Immudyne NQ is well known in the literature for characterization of ß-1,3 1,6-glucans. The data listed in this table are physical constants for the yeast ß-glucan universally. All Immudyne products for the whole history of the company have been tested for homogeneity by carbon-13 n.m.r. to verify both structure and composition. The complement activation ability of the Immudyne NQ is closely correlated with its resonance intensity as well as energy of chemical shift.

CONCLUSIONS

The Immudyne NQ contains far less impurities and is much more uniform in composition than the Myco-Immune product. Although both of these products possess immune-stimulating potential, the complexity of the Myco-Immune product would indicate that it would be potentially problematic to manufacture and store reliably. The amount of ultraviolet absorbing material in the Myco-Immune is highly complex as noted in the liquid chromatography. Although many of the mushroom polysaccharides are indeed typical fungal ß-1,3 1,6-glucans, only a small part of all the carbohydrate in Myco-Immune is this required structure for effective interactions with cell surface lectin and toll receptors of the macrophage to bring about immunopotentiation.

Approved by John R. Vercellotti, Ph.D.
Sharon V. Vercellotti, M.S.

Signature: John R. Vercellotti

Signature: Sharon V. Vercellotti

Immudyne, Inc.