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Yeast-Extract Spreads: History, Raw Materials, Manufacture, Chemistry, Quality Control and the Role of Multinuclear NMR Spectroscopy in Composition Profiling, Consistency Monitoring, Adulteration Detection and Product Identity

With ^{1}H, ^{13}C, DEPT, HSQC, HMBC and 2D J-resolved NMR data on Marmite, Marmite XO, Vegemite, Promite and Sanitarium Marmite

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Abstract

Yeast-extract spreads — Marmite, Marmite XO, Vegemite, Promite and the New Zealand Sanitarium Marmite — are concentrated autolysates of *Saccharomyces* yeast, blended with salt and minor flavoring, coloring, carbohydrate and vitamin ingredients, and they exemplify a much larger industrial category of yeast extracts used as savory flavor bases, umami and kokumi ingredients and cell-culture nutrients. This white paper reviews the history of the products from Liebig and the foundation of the Marmite Food Extract Company in 1902 to the present ownership of the brands, the raw materials (brewer's spent yeast versus primary-grown yeast and the formulation ingredients declared for each product), the manufacturing process (debittering, salt-induced autolysis, plasmolysis, enzymatic hydrolysis, nuclease chemistry, separation, concentration and thermal flavor development), the chemistry of the extracts (peptides and free amino acids, 5′-ribonucleotides and their degradation products, glutathione and γ-glutamyl peptides, trehalose, glycogen and dextrins, organic acids, choline compounds, phosphorus species, vitamins, minerals, Maillard products and biogenic amines) and the conventional quality-control and regulatory framework. Against that background, the principles of ^{1}H, ^{13}C, DEPT, HSQC, HMBC, J-resolved, DOSY, ^{31}P and benchtop NMR as applied to these matrices are set out, and a set of multinuclear NMR spectra acquired at Process NMR Associates on the five products at 300 MHz in D_2O is interpreted region by region. The spectra separate the products by class and by formulation: the British and Australian products share a brewer's-yeast autolysate core of free amino acids (alanine dominant), RNA degradation products, choline compounds and organic acids, and are distinguished by their carbohydrate residue (α-(1→4)/(1→6)-glucan in Marmite, trehalose in Vegemite) and by the depth of nucleolysis (deeper in the 28-day Marmite XO); the New Zealand product carries the same core beneath dominant sucrose signals; and Promite is shown — in ^{1}H, ^{13}C and HSQC alike — to be an acid-hydrolyzed vegetable protein and sucrose based spread, lacking the RNA degradation products and carrying the formate and levulinate markers of acid hydrolysis. The contrast between the British and the Australasian products is examined explicitly: the trehalose of Vegemite is argued to be a retained yeast metabolite rather than an added ingredient, recording the physiological state of the yeast at intake and a process that spared the trehalases, whereas the sucrose of the New Zealand product and of Promite is a deliberate addition whose purpose — sweetness, suppression of bitterness and saltiness, and masking of the potassium chloride used as a salt substitute — is consistent with the taste-interaction literature; the deeper nucleolysis of the British products was read in version 1.1 as the alternative, umami-intensifying route to palatability, a reading that is withdrawn here because the RNA-derived material proves to be present largely as nucleosides. A quantitative NMR analyte panel, a fingerprint-based statistical process control scheme using mean-centered, Pareto-scaled data, a set of process-diagnostic ratios, and NMR strategies for the detection of carbohydrate dilution, HVP substitution, added 5′-ribonucleotides and yeast-source substitution are proposed, together with a candid statement of what NMR cannot determine (added versus native glutamate, volatiles, most vitamins, inorganic salts, biogenic amines). A PNA analytical program is outlined, and the limitations of the present single-lot, unbuffered survey are assessed critically. Version 1.1 incorporated the shift-corrected figure set and the additional Promite ^{13}C and HSQC data supplied by the author in September 2026. In this version 1.2 the gHSQC, gHMBC and 2D J-resolved spectra were recovered numerically from the MestReNova documents and assigned in full: 155 one-bond and 83 long-range ^{1}H–^{13}C correlations and some thirty J-resolved multiplets were assigned, and fifty constituents or structural classes were graded for each product. The assignment establishes eighteen free amino acids, pyroglutamate in amount comparable with glutamate, 4-aminobutyrate and a peptide-bound population; resolves the trimethylammonium singlet into glycerophosphocholine, free choline and, in the two British products only, betaine; shows glycerol to give the most intense ^{13}C line of Marmite XO; characterizes the soluble α-glucan as a branched dextrin accompanied by cell-wall mannan; reassigns the 8.6 ppm singlet from the adenine ring to histidine and the RNA-derived signals largely to nucleosides; confirms monounsaturated fatty acyl chains in Vegemite; and supports levulinate, citrate and formate in Promite. The ^{1}H spectra give about 0.29, 0.08, 1.2 and 0.7 mol of trehalose per mol of free alanine in Marmite, Marmite XO, Vegemite and the New Zealand Marmite, each to about one part in ten, so that the New Zealand product resembles Vegemite, and not the British Marmite, in carrying as much trehalose as

soluble α-glucan or more; about one-eighth of its added sucrose has been inverted. The gHSQC cross-peak volumes were found not to be quantitative between unlike signals and are used for like signals only. Several readings of version 1.1, including the scale of the ^{13}C axes, were corrected in consequence. The document is supported by 72 verified peer-reviewed references and 22 documentary sources, and by a companion workbook of working tables.

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1. Introduction and Scope

Yeast-extract spreads occupy an unusual position among manufactured foods. They are, in essence, the concentrated soluble contents of *Saccharomyces* cells that have been induced to digest themselves, blended with salt and small quantities of flavoring materials, and they have been eaten in largely unchanged form for more than a century. Marmite has been made in Burton upon Trent since 1902, Vegemite in Melbourne since 1923, and New Zealand Marmite in Christchurch for most of the intervening period.[1–3] The products are at once a by-product valorization of the brewing industry, a historically important dietary source of the B-vitamin complex, and a technically demanding matrix in which free amino acids, peptides, 5′-ribonucleotides, sugars, polyols, organic acids, choline compounds, vitamins and roughly ten percent sodium chloride coexist in a dark, viscous, water-based paste.[4–6]

The commercial yeast-extract sector that grew up around these spreads is now far larger than the spreads themselves. Yeast extracts and autolysates are used as savory flavor bases, as sources of glutamate and of the taste-enhancing 5′-nucleotides, as kokumi ingredients, as salt-reduction aids, and as complex nutrient supplements for microbial, insect and mammalian cell culture, where lot-to-lot variability of the extract is a recognized process risk.[4,5,7] Wherever a yeast extract is used, the same analytical questions arise: what is in it, how much, how consistently from lot to lot, and whether the material is what it claims to be.

Nuclear magnetic resonance (NMR) spectroscopy is well suited to these questions because it observes every hydrogen-, carbon- or phosphorus-containing constituent of a solution simultaneously, without extraction, derivatization or compound-specific calibration, and because the resulting signal intensities are directly proportional to molar amounts under quantitative acquisition conditions.[8–10] The present white paper was prepared to place a set of multinuclear NMR data acquired at Process NMR Associates (PNA) on five commercial spreads — Marmite and Marmite XO (Unilever, United Kingdom), Vegemite (Bega Group, Australia), Promite (Mars Food, Australia) and Sanitarium Marmite (Sanitarium Health Food Company, New Zealand) — into the context of the history, raw materials, manufacturing chemistry and quality-control practice of the yeast-extract industry, and to set out how one-dimensional and two-dimensional ^{1}H and ^{13}C NMR, supplemented by ^{31}P NMR and benchtop instrumentation, can be used to characterize component distribution, to monitor quality and consistency, to detect adulteration and to establish product identity.

The document is organized in twelve sections. Sections 2 to 6 review the history of the products, the raw materials, the manufacturing process, the chemistry of the extracts and the conventional quality-control framework. Section 7 reviews the NMR methodology and the limited but instructive literature on NMR of yeast extracts and related hydrolysates. Section 8 presents and interprets the PNA spectra product by product and, in Sections 8.10 to 8.15, which were added in version 1.2, assigns the two-dimensional spectra from the numerical data. Sections 9 and 10 develop the quality-monitoring and authentication applications, Section 11 proposes an analytical program for the PNA laboratory, and Section 12 provides a critical assessment of what the present data set can and cannot support. The spectral interpretation offered in Sections 8.3 to 8.9 is qualitative and is based on the spectra as presented in the source figures, and the assignments of Sections 8.10 to 8.15 rest on the processed numerical data and are at most semi-quantitative; integrated concentrations, replicate acquisitions and chemometric treatment are reserved for a subsequent revision in which the author's own quantitative data will be incorporated.

2. Historical Development of Yeast-Extract Spreads

2.1 Liebig and the Origin of Yeast Extract

The idea that spent brewer's yeast could be converted into a palatable concentrated food is generally attributed to Justus von Liebig, whose work on meat extract in the 1860s established the commercial category of savory extracts and who observed that yeast cells could be concentrated, bottled and eaten in the manner of meat extract.[1,11] The essential chemistry — the release of the cell contents by the yeast's own

enzymes, followed by removal of the insoluble cell walls and concentration of the soluble fraction — was practiced empirically for decades before its biochemical basis was understood. The vitamin era that followed Casimir Funk's work in the 1910s gave the products their second identity, as concentrated sources of the water-soluble B-complex; by 1929 the Marmite Food Extract Company was advertising its product at the British Industries Fair as one of the richest known foods in "the essential Vitamin B".[1] The clinical demonstration by Lucy Wills in 1931 that a yeast extract corrected the macrocytic anemia of pregnancy in Bombay mill workers — the observation that led ultimately to the identification of folic acid — remains the most celebrated single episode in that history.[12]

2.2 Marmite (United Kingdom) and Marmite XO

The Marmite Food Extract Company was formed in 1902 by the retired sugar merchant Frederick Wissler and others, and Burton upon Trent was chosen for the first factory because the town's breweries — Bass above all, then the largest brewer in the world — could supply the surplus yeast on which the process depends.[1] A second factory was opened at Camberwell Green, London, in 1907. The company passed to Bovril in the 1930s; Bovril was acquired by Cavenham Foods in 1971; Marmite Limited was bought by CPC (United Kingdom) in 1990, CPC became Bestfoods in 1998, and Bestfoods merged with Unilever in 2000, under whose ownership the brand remains.[1] The product name is taken from the French word for a lidded cooking pot, an image of which has appeared on the label since the beginning.

The current United Kingdom label declares yeast extract (carrying the cereal allergens barley, wheat, oats and rye), salt, vegetable juice concentrate, the vitamins thiamin, riboflavin, niacin, vitamin B_{12} and folic acid, and natural flavoring containing celery.[13,14] Declared nutrition per 100 g is approximately 1,087 kJ, 34 g protein, 30 g carbohydrate of which 1.2 g sugars, and 10.8 g salt, with thiamin, riboflavin, niacin and folic acid declared at roughly 7.7 mg, 6.8 mg, 69 mg and 1,250 µg per 100 g respectively.[14] The cereal allergen declaration is itself analytically informative: it records that the yeast extract is derived from brewer's yeast that has grown in a cereal wort, a point returned to in the interpretation of the polysaccharide signals in Section 8.

Marmite XO ("extra old") was first sold in March 2010 as a limited edition and has been reissued periodically since. The manufacturer describes it as the original recipe matured four times longer — 28 days rather than the usual seven — to produce a fuller-bodied and stronger-flavored product.[11,15] From the analytical standpoint the interesting question is what a fourfold longer maturation does to the composition: continued proteolytic and nucleolytic action would be expected to raise the free amino acid and free nucleotide fractions, continued Maillard chemistry to deepen color and aroma, and continued glycolytic and hydrolytic action to reduce the residual storage carbohydrate. The PNA spectra of Marmite and Marmite XO discussed in Section 8 allow a first qualitative test of that expectation.

2.3 Marmite in New Zealand (Sanitarium)

The New Zealand Marmite is a different product from the British one, made by a different company to a different recipe, and the two are best regarded as distinct members of the same family. The Sanitarium Health Food Company records that British Marmite first arrived in New Zealand by ship in 1910, that Sanitarium obtained rights to the product — the company's own materials variously cite 1908 for distribution rights and 1919 for exclusive rights — and that in the 1930s and 1940s the company experimented with British Marmite and local ingredients until it was manufacturing its own New Zealand Marmite in Christchurch.[3,16] The Christchurch factory was gutted by fire in 1966 and rebuilt.

Production at the Papanui (Christchurch) factory was suspended in March 2012 because a cooling tower damaged in the 22 February 2011 Canterbury earthquake had rendered the Marmite building unsafe; further damage was found during repair, retail stocks were exhausted, and the product did not return to shelves until 20 March 2013, an episode that acquired the popular name "Marmageddon".[3,17,18] The current declared ingredients are yeast, sugar, salt, mineral salt (potassium chloride), color (caramel III), corn maltodextrin,

mineral (iron), the vitamins niacin, thiamin, riboflavin, folate and B_{12}, herbs and spices, with a statement that the product contains derivatives of barley and wheat.[16] Declared sodium is 0.166 g and potassium 0.14 g per 5 g serving, corresponding to roughly 3.3 g sodium and 2.8 g potassium per 100 g.[19] The presence of sucrose, maltodextrin, potassium chloride, caramel and iron distinguishes this formulation sharply from the British product, and, as will be seen, each of the carbohydrate additions is immediately visible by NMR.

2.4 Vegemite (Australia)

Vegemite was developed in Melbourne by the chemist Cyril Percy Callister for the Fred Walker Company, which had been unable to obtain Marmite after the First World War. Callister applied autolysis to the waste yeast of the Carlton and United brewery, concentrated the clarified extract and blended it with salt and with celery and onion extracts to produce a black paste that was first sold in 1923 under a name chosen by public competition.[2,20] Sales were poor against the entrenched Marmite, and from 1928 to 1935 the product was marketed as "Parwill" — the pun being "Marmite but Parwill" — before reverting to Vegemite. Fred Walker had formed the Kraft Walker Cheese Company with J. L. Kraft in 1925, and after Walker's death in 1935 Vegemite was promoted by Kraft with free samples and poetry competitions. In 1939 it was endorsed by the British Medical Association as a vitamin-B-rich food, it was purchased in bulk for the armed forces during the Second World War, and by the late 1940s it was reported to be in nine of ten Australian households.[2,20] The brand passed through Kraft and Mondelēz International and was purchased by the Bega Group in January 2017 as part of a US$460 million transaction that returned it to Australian ownership.[2,20] It continues to be made at the Port Melbourne site.

The current declared ingredients are yeast extract (from yeast grown on barley and wheat), salt, mineral salt (508, potassium chloride), malt extract (from barley), color (150c), flavors, niacin, thiamine, riboflavin and folate.[21] The manufacturer's nutrition panel gives, per 100 g, 729 kJ, 25.9 g protein, less than 1 g fat, 11.1 g carbohydrate of which 2.4 g sugars, 3,300 mg sodium, 11 mg thiamin, 8.6 mg riboflavin, 50 mg niacin and 2,000 µg folate.[22] A reduced-sodium variant introduced in 2014, a gluten-free variant made from yeast grown on non-gluten feedstocks, and a cheese-blended variant complete the current range.[20,23] A proteomic study of the retail product identified abundant intracellular yeast proteins and barley proteins in both Vegemite and Marmite, a direct molecular record of the brewing origin of the yeast.[24]

2.5 Promite and the Vegetable-Extract Category

Promite was developed by Henry Lewis and Company in Australia in the 1950s and passed to Mars in 1967 with the acquisition of the MasterFoods business; it has always been positioned as a sweeter, milder alternative to Vegemite.[25,26] The product is now labeled a "vegetable extract spread" rather than a yeast extract, and the current declaration lists vegetable protein extract (39 percent), sugar, yeast extract, water, color (caramel 150c), cornflour (wheat), salt, glucose syrup, onion, emulsifier (glycerol monostearate), thickeners (modified cornstarch, carrageenan), acidity regulator (citric acid) and spice extract, with 13.3 g protein, 32.9 g carbohydrate, 18.1 g sugars, 2.7 g fat and 4,575 mg sodium per 100 g.[27,28] An earlier formulation is recorded as having listed hydrolyzed vegetable protein extract first, and the manufacturer removed added B vitamins and flavor enhancers from the recipe in 2013.[25,26] Promite is therefore best described as a hydrolyzed-vegetable-protein and sucrose based savory spread that contains yeast extract as a minor component, and it serves in this paper as a valuable negative control against which the yeast-derived products can be compared.

2.6 Other Products and the Industrial Yeast-Extract Sector

Yeast-extract spreads exist in most countries with a British culinary inheritance or a large brewing industry: Vegex in the United States (since 1913), Cenovis in Switzerland, Vitam-R in Germany, Viandox in France, AussieMite and OzEmite in Australia, and numerous retailer own-label products in the United Kingdom.[11] Far larger in tonnage is the industrial sector that supplies yeast extracts, autolysates, nucleotide-enriched extracts and glutathione-enriched extracts to the processed-food, flavor and biotechnology industries;

production is dominated by specialty-yeast companies that grow baker's-type *Saccharomyces cerevisiae*, and in some cases *Cyberlindnera* (*Candida*) utilis or *Kluyveromyces* species, on molasses or other substrates specifically for extraction, rather than relying on brewery surplus.[4–6] The compositional consequences of the choice between brewer's spent yeast and primary-grown yeast are discussed in Section 3 and are directly relevant to the NMR-based identity questions treated in Section 10.

3. Raw Materials

3.1 Yeast Biomass: Brewer's Spent Yeast versus Primary-Grown Yeast

Brewer's spent yeast is the second largest by-product of brewing after spent grain, and its use as the feedstock for yeast extract is the historical basis of the spread products.[29] The yeast recovered from a fermenter is not a clean biomass: it is a slurry in beer, it carries adsorbed hop resins and iso-α-acids that make it bitter, it retains beer dextrins, proteins and polyphenols in the interstitial liquid, and its physiological state depends on the number of generations it has been re-pitched, on the original gravity of the wort and on the time of cropping.[29,30] In a pilot study in which the cropping time, original gravity and yeast strain were varied systematically, every measured attribute of the resulting extract — total protein, fat, ash, the proteinogenic amino acid spectrum, the folate vitamers and the antioxidant activity — was found to depend on the composition of the spent yeast, leading the authors to conclude that the biodiversity of the spent yeast directly affects the physiologically valuable content of the extract.[30] Serial re-pitching was likewise shown to influence the yield of flavor-enhancer 5′-nucleotides obtainable from spent yeast.[31]

Primary-grown yeast, by contrast, is cultivated aerobically on molasses or other defined feedstocks under conditions chosen to maximize biomass, protein or specific metabolites, and it carries none of the hop, dextrin or cereal-protein burden of brewery yeast.[4,6] A review of the industrial literature places the protein content of yeast extracts between 14 and 64 percent of dry weight, the free glutamic acid between 0.5 and 17.5 g per 100 g, free alanine between 3.7 and 26.6 g per 100 g, total nucleic acid at up to 15 percent, trehalose near 1 percent, β-glucan at up to 27 percent in wall-containing preparations, potassium between 1 and 10 g per 100 g and phosphorus at up to 3.4 g per 100 g, ranges so wide that they are best read as a statement that "yeast extract" is a family of products rather than a substance.[4] The same conclusion was reached in a brewing-oriented review, which found free amino acids to constitute anywhere from 10 to 77.5 percent of the proteinogenic material in commercial extracts, total carbohydrate from 8.5 to 20.6 percent, and B-vitamin contents that were detectable in some products and absent in others.[32,33]

For the spread products the distinction matters in two directions. The brewery origin of the yeast contributes materials that a primary-grown extract does not contain — barley proteins and α-glucan dextrins, hop-derived bitter acids, and the maltose and maltotriose residues of an attenuated wort — and these are potential identity markers.[24,29] Conversely, molasses-grown yeast can carry betaine and other feedstock-derived solutes that brewer's yeast does not, so that the small-molecule inventory of a spread made from primary-grown yeast extract would be expected to differ from one made from brewery yeast even when the autolysis conditions are identical.

3.2 Composition of the Yeast Cell Relevant to Extract Chemistry

A *Saccharomyces* cell at harvest is, on a dry basis, roughly 40 to 55 percent protein, 5 to 12 percent RNA with a much smaller DNA fraction, 25 to 35 percent carbohydrate, 4 to 7 percent lipid and 5 to 10 percent ash, the exact proportions depending on strain, growth phase and nutrient status.[4,6] Each of these fractions has a distinct fate during extract manufacture and a distinct NMR signature.

The protein is converted by the yeast's own vacuolar proteinases and peptidases, or by added proteases, into a mixture of oligopeptides, small peptides and free amino acids, and the degree to which that hydrolysis is carried determines the "free amino nitrogen" of the extract, its glutamate content and its taste.[6,32] The RNA is hydrolyzed by endogenous ribonucleases through 2′,3′-cyclic nucleotide intermediates to 3′- and 2′-

nucleotides, nucleosides and free bases, none of which carry the umami-enhancing activity of the 5′-ribonucleotides, so that an extract intended to deliver 5′-inosinate and 5′-guanylate must be made by a route in which the endogenous nucleases are inactivated and an exogenous 5′-phosphodiesterase is used instead.[34,35] The carbohydrate comprises the cell-wall polysaccharides — β-(1→3)/β-(1→6)-glucan and the highly branched α-mannan of the mannoproteins — and the two intracellular reserves, glycogen and trehalose, which in stressed or stationary-phase brewer's yeast can each amount to 10 to 20 percent of the dry mass.[36–38] The wall polysaccharides are largely removed with the cell debris, but soluble mannoprotein fragments, partially degraded glycogen and trehalose that has escaped hydrolysis by trehalase can all appear in the extract. The lipid fraction, predominantly phospholipid, is degraded during autolysis to glycerophosphocholine, choline, phosphate and fatty acids, which is why yeast extracts are notable sources of choline compounds. The ash fraction is dominated by potassium and phosphate, with magnesium, and the phosphate is present both as orthophosphate and as inorganic polyphosphate, a vacuolar reserve that is characteristically visible in ^{31}P NMR spectra of intact yeast.[39,40] The vitamins of interest — thiamin, riboflavin, niacin (as nicotinic acid and nicotinamide, including the nucleotide-bound forms), pantothenic acid, pyridoxine and the folate vitamers — are present natively at levels that make brewer's yeast one of the richest natural sources of the B-complex, and they are supplemented in the spread products to declared label values.[5,30]

3.3 Formulation Ingredients

The formulation ingredients declared for the five products studied here are summarized in Table 1. Sodium chloride is common to all and serves as preservative, as plasmolysis agent in the traditional process, and as a flavor component; the declared salt content of the British product, 10.8 g per 100 g, corresponds to approximately 185 mmol of chloride per 100 g, a quantity that matters for NMR probe tuning as discussed in Section 7.[14,41] Vegetable juice concentrates, celery and onion extracts, herbs and spices contribute color and aroma at low levels and are analytically minor. Malt extract, declared for Vegemite, contributes maltose, maltotriose and higher dextrins together with barley proteins and additional color. Sucrose, glucose syrup and maltodextrin, declared for the New Zealand Marmite and for Promite, contribute sweetness and body and dominate the carbohydrate region of the corresponding NMR spectra. Caramel color (150c and 150d, "caramel III" and "caramel IV") is used in the Australasian products; it is a heterogeneous, high-molecular-mass Maillard-type polymer that contributes little sharp NMR signal but can contribute to the broad baseline hump. Potassium chloride, declared as a mineral salt in the Australasian products, is invisible to ^{1}H NMR. Iron, declared for the New Zealand product, is paramagnetic and is a potential source of line broadening. The vitamins, added to declared label values, are present at milligram-per-100-g levels that place most of them at or below the practical detection limit of a routine ^{1}H spectrum, with niacin as the exception discussed in Section 8.

Table 1. Declared ingredients and selected declared nutrition values for the five products examined. Ingredient lists are given as declared on current retail labels, rendered in American spelling; the order is the declared descending order by weight.

Product (manufacturer, country)	Declared ingredients	Selected declared values per 100 g
Marmite (Unilever, UK)	Yeast extract (contains barley, wheat, oats, rye), salt, vegetable juice concentrate, vitamins (thiamin, riboflavin, niacin, vitamin B_{12}, folic acid), natural flavoring (contains celery)	Energy 1,087 kJ; protein 34 g; carbohydrate 30 g (sugars 1.2 g); salt 10.8 g; thiamin 7.7 mg; riboflavin 6.8 mg; niacin 69 mg; folic acid 1,250 µg
Marmite XO (Unilever, UK)	Original Marmite recipe, matured four times longer (28 days)	Not separately tabulated; assumed comparable to Marmite

Product (manufacturer, country)	Declared ingredients	Selected declared values per 100 g
Vegemite (Bega Group, Australia)	Yeast extract (from yeast grown on barley and wheat), salt, mineral salt (508), malt extract (from barley), color (150c), flavors, niacin, thiamine, riboflavin, folate	Energy 729 kJ; protein 25.9 g; fat <1 g; carbohydrate 11.1 g (sugars 2.4 g); sodium 3,300 mg; thiamin 11 mg; riboflavin 8.6 mg; niacin 50 mg; folate 2,000 µg
Sanitarium Marmite (Sanitarium Health Food Co., New Zealand)	Yeast, sugar, salt, mineral salt (potassium chloride), color (caramel III), corn maltodextrin, mineral (iron), vitamins (niacin, thiamin, riboflavin, folate, B_{12}), herbs, spices; contains derivatives of barley and wheat	Sodium ≈ 3,300 mg; potassium ≈ 2,800 mg (from 0.166 g and 0.14 g per 5 g serving)
Promite (Mars Food, Australia)	Vegetable protein extract (39 %), sugar, yeast extract, water, color (caramel 150c), cornflour (wheat), salt, glucose syrup, onion, emulsifier (glycerol monostearate), thickeners (modified cornstarch, carrageenan), acidity regulator (citric acid), spice extract	Energy 920 kJ; protein 13.3 g; fat 2.7 g; carbohydrate 32.9 g (sugars 18.1 g); sodium 4,575 mg

Sources for Table 1: Marmite,[13,14] Marmite XO,[15] Vegemite,[21,22] Sanitarium Marmite,[16,19] and Promite.[27,28]

4. Manufacturing Process

4.1 Debittering and Washing of Spent Yeast

Spent brewer's yeast is received as a slurry of 10 to 20 percent solids in beer. It is screened to remove trub and hop fragments, washed with water to remove entrained beer, and debittered by washing with dilute alkali (typically sodium hydroxide or sodium carbonate at pH 8 to 10) or with dilute salt solution, which desorbs the hop iso-α-acids and other bitter resins from the cell surface; the yeast is then re-acidified and either processed immediately or dried.[6,29] Washing also removes a portion of the soluble beer dextrins and proteins, but not all of them, and the residual cereal material is retained through the subsequent steps, which is the reason that barley proteins are detectable in the finished spreads and that cereal allergens must be declared.[14,24] Manufacturers of the spreads describe their processes only in outline; the New Zealand producer states that the product is made by concentrating yeast and blending it with herbs and spices, and the Australian producer that the original process applied autolysis to brewery yeast followed by concentration of the clarified extract and blending with salt and vegetable extracts.[2,16] What follows is therefore the generic process as documented in the industrial and academic literature.

4.2 Cell Disruption: Autolysis, Plasmolysis, Enzymatic Hydrolysis and Mechanical Methods

Autolysis is the self-digestion of the yeast cell by its own hydrolytic enzymes once the membrane integrity that normally sequesters them is lost. In the industrial process a yeast cream of 12 to 15 percent solids is brought to 45 to 55 °C at pH 5.0 to 6.5 and held for 12 to 50 hours with gentle agitation; the temperature is chosen to kill the cell and to accelerate the endogenous proteinases, peptidases, nucleases, glucanases and lipases without denaturing them.[4,6,32] A controlled temperature shock combined with osmotic stress from added sodium chloride was found to be particularly effective, and this combination is the basis of the traditional spread process, in which salt is added to the yeast suspension to create a hypertonic medium that shrinks the cells and initiates their breakdown before the mass is heated.[11,32] Autolysis can also be promoted by ethanol, by saponins and by other membrane-active agents.

Plasmolysis in the industrial sense refers to the use of an organic solvent, most commonly 1.5 percent ethyl acetate, to permeabilize the membrane; it typically gives a higher extract yield than salt-induced autolysis and requires the solvent to be stripped from the product.[4,42] Enzymatic hydrolysis uses added proteases —

papain, bromelain, alkaline bacterial proteases such as Alcalase, or fungal protease blends — at 48 to 72 hours, 50 to 58 °C and pH 5 to 7, and yields the highest recoveries of solids and protein; in a direct comparison of the three routes on baker's yeast, plasmolysis was more efficient than autolysis, and enzymatic hydrolysis with 0.2 percent Alcalase released the most solids and protein.[42] Mechanical disruption by bead milling, high-pressure homogenization or ultrasonication releases the cell contents without hydrolysis and is used where an unhydrolyzed protein fraction is wanted, but it was found to produce far lower free amino acid contents than autolysis — a maximum of roughly 200 mg per gram of extract for non-*Saccharomyces* strains against 340 mg per gram for commercial *Saccharomyces* strains under autolytic conditions, and 101 to 449 mg per gram across the full set of conditions studied.[30,32] The 21 CFR definition of bakers yeast extract as the concentrated solubles of "mechanically ruptured" cells reflects the regulatory origin of that product category rather than the dominant industrial practice.[43]

The disruption route is the single largest determinant of extract composition. The free amino acid content, the peptide size distribution, the glutamate content, the bitterness (which arises from hydrophobic peptides and is reduced by extended peptidase action or by microbial debittering), the residual carbohydrate, the fraction of RNA that survives as oligonucleotide and the vitamin retention all differ between routes.[4,32,44] For the analytical chemist this means that a product's NMR fingerprint is in large part a record of its process, and that a change of process conditions — a shorter or longer autolysis, a different temperature, an added enzyme — will be visible in the spectrum before it is visible in any single conventional specification parameter.

4.3 Nucleotide Chemistry During Processing

The nucleotide chemistry deserves separate treatment because it determines whether an extract is merely savory or actively taste-enhancing. Yeast RNA is degraded during ordinary autolysis by endogenous ribonucleases of the RNase T_2 type, which cleave the phosphodiester chain through 2′,3′-cyclic phosphate intermediates to give 3′-nucleotides and, after further action of phosphatases, nucleosides and free purine and pyrimidine bases.[34] The 2′,3′-cyclic nucleotides are sufficiently long-lived to be observed directly: a ^{31}P NMR study of a standard bacteriological medium found high levels of adenosine, cytidine, guanosine and uridine 2′,3′-cyclic monophosphates in the yeast-extract component, with characteristic resonances at 20 to 21 ppm, and reproduced their formation both during the autolysis of *S. cerevisiae* cells and in model reactions of ribonuclease with RNA.[34] None of these species is umami-active.

The taste-active 5′-ribonucleotides — disodium 5′-inosinate (IMP) and disodium 5′-guanylate (GMP), whose flavor-enhancing properties were described by Kuninaka in 1960 and whose synergy with glutamate was quantified by Yamaguchi in 1967 — are obtained from yeast RNA only when the endogenous nucleases are first inactivated by heat and the RNA is then hydrolyzed by an exogenous 5′-phosphodiesterase, classically from *Penicillium citrinum*, followed by conversion of 5′-adenylate to 5′-inosinate by adenylate deaminase.[35,45] The molecular basis of the synergy is now understood: glutamate binds in the hinge region of the Venus-flytrap domain of the T1R1 subunit of the umami receptor and the 5′-nucleotide binds at an adjacent site near the opening of the flytrap, stabilizing the closed conformation.[46] Nucleotide-enriched extracts containing several percent of IMP plus GMP are important industrial products; conventional spread products made by salt-induced autolysis would be expected to contain a mixture of 3′- and 5′-nucleotides, nucleosides, cyclic nucleotides and free bases in proportions that record the process history, and to contain relatively little 5′-IMP because the deaminase step is absent. NMR is unusually well placed to read that record, because the 2′-, 3′-, 5′- and 2′,3′-cyclic phosphate esters of the same nucleoside are resolved from one another in ^{31}P NMR and give distinguishable H-1′ and H-8/H-2 resonances in ^{1}H NMR.[34]

4.4 Separation, Concentration, Thermal Flavor Development and Formulation

After disruption the digest is pasteurized to inactivate the enzymes and is separated by centrifugation or decanter, the cell-wall fraction being sieved out or centrifuged off and in modern plants recovered as a glucan-mannan product in its own right.[4,6] The clarified extract, at 8 to 15 percent solids, is concentrated in

multiple-effect or falling-film evaporators to a paste of roughly 70 to 80 percent solids for the spread products, or spray-dried to a powder for industrial extracts; the concentration step is also a thermal step in which Maillard reactions between the free amino acids, peptides and reducing sugars, and Strecker degradation of the amino acids, develop the characteristic dark color and the meaty, roasted aroma.[6,44,47] Salt is added during or after autolysis, the vegetable, spice, malt or caramel components and the vitamin premix are blended in, and the paste is hot-filled into jars. The heat history is a critical quality variable: a systematic study of thermal treatment found that non-volatile taste compounds decreased and volatile aroma compounds increased with temperature, that the aroma at 100 to 110 °C was buttery, nutty and meaty, and that treatment at 140 °C produced a strong sour and sulfurous odor together with bitterness, so that the process window is narrow.[44]

4.5 Process Variables and Their Compositional Consequences

Table 2 collects the principal process variables with their expected compositional consequences and the NMR-observable quantities through which each consequence can be followed. The table is intended as a bridge between the process chemistry of this section and the analytical program of Sections 9 to 11.

Table 2. Process variables in yeast-extract manufacture, their compositional consequences and the corresponding NMR-observable quantities.

Process variable	Compositional consequence	NMR-observable quantity
Yeast source (brewer's spent vs primary-grown; strain; generations)	Residual dextrins, barley proteins and hop acids in brewer's yeast; betaine and molasses solutes in primary yeast; trehalose and glycogen reserves depend on physiological state	α-(1→4)-glucan anomeric envelope (δH ≈ 5.4, δC ≈ 100); trehalose H-1 (δH 5.19) and C-1 (δC ≈ 94–96); betaine singlets (3.27, 3.90); maltose/maltotriose reducing-end signals
Debittering (alkali or salt wash)	Removal of iso-α-acids and adsorbed beer solids; loss of some soluble carbohydrate	Aromatic/olefinic hop-acid signals absent or present; dextrin envelope intensity
Disruption route (salt autolysis, ethyl acetate plasmolysis, protease, mechanical)	Free amino acid fraction 10–78 % of nitrogen; peptide size distribution; bitterness; residual ethyl acetate	Free amino acid α-CH and side-chain signals; peptide backbone envelope 4.2–4.6 and 8.0–8.5 (in H_2O); ethyl acetate singlet 2.07/quartet 4.13; DOSY separation of peptides
Autolysis temperature, pH and time	Extent of proteolysis and nucleolysis; trehalase and glucoamylase action on reserves; glutaminase/asparaginase conversion of Gln/Asn to Glu/Asp; GABA formation by glutamate decarboxylase	Glu/Gln and Asp/Asn ratios; GABA (3.01, 2.30, 1.90); trehalose and glycogen intensities; nucleoside/base versus nucleotide ratios
Nuclease strategy (endogenous RNase vs heat-inactivation plus 5′-phosphodiesterase and deaminase)	3′-nucleotides, 2′,3′-cyclic nucleotides, nucleosides and bases versus 5′-IMP and 5′-GMP	^{31}P: 2′,3′-cyclic ≈ 20–21 ppm; 5′-, 3′-, 2′-monoesters resolved near 0–5 ppm; ^{1}H: H-8 and H-1′ resonances 8.1–8.6 and 5.9–6.2 ppm
Pasteurization and evaporation (time–temperature)	Maillard and Strecker chemistry; loss of Lys ε-amine and reducing sugars; color; furanones, pyrazines, sulfur volatiles	Reducing-sugar anomeric signals; formate and acetate; broad baseline from melanoidins; 5-HMF (9.45 s, 7.5 d, 6.7 d)
Salt addition (NaCl, KCl)	Ionic strength; water activity; preservation	Not directly observed in ^{1}H; affects tuning, pulse width and sensitivity; ^{23}Na and ^{35}Cl NMR possible
Formulation additions (sucrose, glucose syrup, maltodextrin, malt extract, caramel, vitamins, iron)	Dilution of yeast-derived solutes; added carbohydrate; paramagnetic broadening; vitamin fortification	Sucrose (5.41 d, 4.22 d; 104, 93 ppm); glucose α/β anomers; maltodextrin (5.4 br, 100.5); niacin (8.9, 8.6, 8.25, 7.5); line-width and T_1 changes

5. Chemistry of Yeast Extracts

5.1 The Nitrogen Fraction: Peptides and Free Amino Acids

Nitrogenous material is the largest fraction of a yeast-extract spread and is the fraction most directly shaped by the process. The label protein values of 34 g per 100 g for Marmite and 25.9 g per 100 g for Vegemite are Kjeldahl or Dumas nitrogen multiplied by a conventional factor and therefore include peptide, free amino acid, nucleotide, ammonium and choline nitrogen indiscriminately, a limitation that has been pointed out repeatedly in the yeast-extract literature.[5,32] In extracts made by autolysis the free amino acid pool is typically dominated by alanine, glutamate, leucine, lysine, valine, glycine, arginine and proline, with the branched-chain amino acids, alanine, methionine and phenylalanine found to be predominantly (about 85 percent) in the free state, whereas asparagine, glycine and arginine were only 14 to 37 percent free, the remainder being bound in peptides.[32] Glutamine and asparagine are progressively deamidated during autolysis and thermal processing, which raises free glutamate and aspartate and releases ammonia; glutamate may be further decarboxylated to γ-aminobutyrate (GABA) by the yeast glutamate decarboxylase, and an autolysis protocol at 37 °C for 72 hours with added glucose and glutamate approximately doubled the GABA content of the extract.[30] The peptide fraction ranges from dipeptides to oligopeptides of 2,000 to 3,000 Da; hydrophobic peptides rich in leucine, isoleucine, valine, phenylalanine and tyrosine are the principal source of bitterness.[4,32]

From the taste standpoint the free glutamate is the single most important constituent, and its level — 0.5 to 17.5 g per 100 g across the industrial range, and commonly 5 to 8 g per 100 g in flavor-grade extracts — is what makes the products savory.[4,5] The European industry association emphasizes that this glutamate is a natural constituent of the extract and is not the same as added monosodium glutamate, a distinction that is meaningful for labeling but not, as discussed in Section 10, one that solution NMR can make directly.[48]

5.2 Nucleotides, Nucleosides and Purine Bases

The RNA-derived fraction of an autolysate comprises 5′-, 3′- and 2′-mononucleotides, 2′,3′-cyclic nucleotides, nucleosides (adenosine, guanosine, inosine, uridine, cytidine) and free bases (adenine, guanine, hypoxanthine, uracil, cytosine), in proportions set by the nuclease and phosphatase activities that were operative and by the thermal history.[4,34] In the NMR study of hydrolysates for cell culture media, nucleosides were found to be markedly more prominent in two of the five yeast-based products than in the others, illustrating how process differences are recorded in this fraction.[7] Only the 5′-purine nucleotides — 5′-IMP, 5′-GMP and, with lower potency, 5′-AMP — enhance umami; the sensory synergy between glutamate and 5′-inosinate was quantified as early as 1967 and the ranking of the taste intensities of individual amino acids and 5′-nucleotides was established shortly afterward.[35,45] Extracts that are deliberately enriched in 5′-nucleotides by the phosphodiesterase route can contain several percent of IMP plus GMP, and their use in place of, or together with, added disodium inosinate and guanylate is a major commercial application.[4] The total purine load of yeast products is high enough to be relevant to individuals managing hyperuricemia, a point noted in reviews of the category.[4]

5.3 Glutathione and γ-Glutamyl Peptides: Kokumi

Yeast is one of the richest natural sources of the tripeptide glutathione (γ-Glu-Cys-Gly), which ordinary strains accumulate to roughly 0.5 to 1 percent of dry cell mass and which can be raised substantially by strain selection and cultivation under oxidative stress; glutathione-enriched yeast extracts are marketed specifically as kokumi ingredients.[4,49] Kokumi — the sensation of mouthfulness, continuity and complexity that γ-glutamyl peptides impart to savory foods — has been shown to be mediated by the calcium-sensing receptor, at which glutathione and γ-Glu-Val-Gly are agonists.[50] A sensomics study of a commercial yeast extract identified ten kokumi-active peptides — glutathione itself, γ-Glu-Leu, γ-Glu-Val, γ-Glu-Tyr and six leucyl dipeptides (Leu-Lys, Leu-Gln, Leu-Ala, Leu-Glu, Leu-Thr and Ala-Leu) — and found that the bitter

leucyl dipeptides imparted kokumi at concentrations below their bitterness thresholds.[51] Glutathione is readily observed by ^{1}H NMR through its cysteinyl β-CH_2 (2.95 ppm), γ-glutamyl γ-CH_2 (2.55 ppm) and glycyl CH_2 (3.78 ppm) resonances, and its oxidation state (reduced versus disulfide) is also NMR-accessible, so that the kokumi fraction is in principle an NMR-monitorable quality attribute.[52]

5.4 Carbohydrates: Trehalose, Glycogen, Dextrins, Mannan and β-Glucan

Trehalose (α-D-glucopyranosyl-(1↔1)-α-D-glucopyranoside) is the yeast's principal stress-protective and reserve disaccharide, accumulating to 10 to 20 percent of dry mass in stationary-phase and stressed cells and in the yeast used for high-gravity brewing; glycogen, the branched α-(1→4)/α-(1→6)-glucan, is the other reserve and is mobilized rapidly on re-pitching.[37,38] Both are hydrolyzed during autolysis — trehalose by the neutral and acid trehalases, glycogen by glycogen phosphorylase and glucoamylase — but neither hydrolysis goes to completion under all conditions, and industrial extracts are reported to contain trehalose at about 1 percent.[4] The dextrins of the brewing wort (α-(1→4)-linked glucose oligomers with α-(1→6) branch points, from maltotetraose upward, that the brewing yeast cannot ferment) are carried into a brewer's-yeast extract with the entrained beer and, in Vegemite, are additionally supplied by the declared malt extract. Trehalose and the α-glucans are the most diagnostic carbohydrate signals in the spectra of the yeast-derived spreads, and their ^{1}H and ^{13}C assignments are well established: the trehalose anomeric proton is a doublet near 5.19 ppm ($^{3}J \approx 3.8$ Hz) with C-1 near 94 ppm on the tetramethylsilane scale (about 96 ppm relative to DSS), and the α-(1→4)-glucan anomeric proton is a broad signal near 5.4 ppm with C-1 near 100.5 ppm, the α-(1→6) branch anomeric proton appearing near 4.97 ppm with C-1 near 99 ppm.[53–55]

The cell-wall polysaccharides are largely removed with the insoluble debris, but the soluble mannoprotein fraction, whose mannan is a highly branched α-(1→6)-backbone polymer with α-(1→2)- and α-(1→3)-linked side chains and phosphodiester-linked mannose, partitions into the extract to a variable extent; its NMR spectrum is dominated by anomeric signals between 5.0 and 5.4 ppm and by C-1 resonances between 98 and 103 ppm.[36] The β-(1→3)/β-(1→6)-glucan is insoluble and is not expected in a clarified extract, but wall-containing autolysates and "whole-cell" products contain it at up to 27 percent, and its anomeric proton near 4.5 to 4.8 ppm and C-1 near 103 ppm would identify such products.[4,36]

5.5 Organic Acids, Polyols, Choline Compounds and Phosphorus Species

The small-molecule fraction of an autolysate contains the metabolic intermediates and end-products that were present in the cell at harvest together with those formed during processing. Succinate, acetate, lactate, pyruvate, citrate, malate and formate are the organic acids most commonly reported; succinate and acetate are normal yeast metabolites, lactate at elevated levels may indicate bacterial contamination of the spent yeast, and formate is a marker of thermal or acid degradation of sugars.[7,30] Glycerol, the principal osmolyte and redox sink of the brewing yeast, and residual ethanol from the beer are usually present. The phospholipid-derived compounds choline and glycerophosphocholine, together with free phosphate, are characteristically abundant, and ^{31}P NMR of intact yeast has long been used to observe orthophosphate, sugar phosphates, nucleoside di- and tri-phosphates and the vacuolar polyphosphate pool that survives, at least in part, into the extract.[34,39,56] In the metabolomic study of hydrolysates, 90 unique metabolites were identified by NMR across nine products, with only 15 common to all of them, and the identified metabolites accounted for up to 43 percent of the mass of the yeast extracts, a substantially higher proportion than in any plant hydrolysate.[7]

5.6 Vitamins, Minerals and Salt

Brewer's yeast is among the richest natural sources of thiamin, riboflavin, niacin, pantothenate, pyridoxine, biotin and folate, and the extracts retain most of these in soluble form; folate in spent-yeast extracts was measured at 800 to 1,400 µg per 100 g distributed among the 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, 10-formylfolate, tetrahydrofolate and folic acid vitamers, and the distribution was found to depend on the disruption method.[30,33] The spread products are additionally fortified to declared

label values. Vitamin B_{12} does not occur in yeast and is present in Marmite and in the New Zealand product only through fortification.[11] The mineral fraction of a brewer's-yeast extract is dominated by potassium (of the order of 9 g per 100 g in one commercial extract), phosphorus, magnesium and sodium, with zinc, iron and manganese as the principal trace elements.[33,40] Sodium chloride added in formulation raises the salt content of the spreads to 8 to 11 g per 100 g, and potassium chloride is used in the Australasian products as a partial replacement.

5.7 Maillard Chemistry and Aroma-Active Volatiles

The aroma of yeast-extract pastes is generated largely during concentration and pasteurization by Maillard and Strecker chemistry acting on a substrate that is unusually rich in free amino acids, reducing sugars, cysteine and thiamin. Gas chromatography–olfactometry of three commercial pastes identified sulfur compounds, Strecker aldehydes, furanones and pyrazines among the key odor-impact compounds, and later gas chromatography–olfactometry work on commercial pastes has confirmed the dominance of Strecker aldehydes, diketones, pyrazines, furanones and sulfur compounds in the meaty, roasted aroma.[44,47] The interplay between the volatile and non-volatile fractions during heating — the consumption of taste-active amino acids, peptides and nucleotides as aroma is formed — was demonstrated systematically for extracts treated between 25 and 140 °C.[44] These volatiles are present at nanogram to microgram per gram levels and are not the province of NMR, which observes the non-volatile precursors and the non-volatile Maillard products (5-hydroxymethylfurfural, formate, the loss of lysine ε-amino signal into Amadori products, and the broad melanoidin baseline) instead. The two techniques are complementary rather than competing.

5.8 Biogenic Amines and Other Safety-Relevant Constituents

Yeast extracts, and Marmite in particular, hold a place in the pharmacological literature as the foods whose interaction with monoamine oxidase inhibitors was first explained. Blackwell and Marley showed in 1966 that yeast extracts and their constituents, principally tyramine, provoked the hypertensive "cheese reaction" in patients taking these drugs, and the tyramine content of yeast-extract spreads — reported at up to a few milligrams per gram in the early analyses, and lower and highly variable in later surveys — has been re-examined in reviews that place it in the context of realistic serving sizes.[57,58] Histamine, putrescine and cadaverine may also be formed by bacterial contaminants of the spent yeast, and biogenic-amine analysis is therefore a legitimate quality-control parameter for the raw material. Tyramine is observable by ^{1}H NMR through its aromatic AA′BB′ pattern at 6.9 and 7.2 ppm and its ethylamine CH_2 signals near 2.9 and 3.2 ppm, but at milligram-per-gram levels and in the presence of tyrosine, whose aromatic signals coincide, it is not separable at 300 MHz without 2D or chromatographic support; liquid chromatography remains the method of choice for the amines. Purines (Section 5.2) and, for products containing hydrolyzed vegetable protein, the chloropropanols 3-MCPD and 1,3-DCP that are formed during acid hydrolysis of lipid-containing protein sources complete the list of safety-relevant constituents; a 3-MCPD survey of food ingredients from United Kingdom producers and ingredient suppliers established the analytical framework for the latter.[59]

6. Quality Control and Regulatory Context

6.1 Regulatory Definitions

In the United States, bakers yeast extract is affirmed as generally recognized as safe under 21 CFR 184.1983, which defines it as the food ingredient resulting from concentration of the solubles of mechanically ruptured cells of a selected strain of *Saccharomyces cerevisiae*, sets heavy-metal limits (arsenic, cadmium, lead, mercury, selenium and zinc on a dry basis) and microbiological limits (aerobic plate count below 10,000 per gram, yeasts and molds below 10 per gram, negative for *Salmonella*, *Escherichia coli*, coagulase-positive staphylococci and *Clostridium*), and limits its use as a flavoring agent and adjuvant to 5 percent of the food.[43] Yeast extracts derived from brewer's yeast, and autolysates, are

handled under the general provisions for food ingredients. In the European Union, yeast extract is likewise a food ingredient rather than an additive, is labeled by name, and carries no E-number; the European Association for Specialty Yeast Products describes it as the product obtained when the contents of the yeast cell are broken down by enzymes and the cell wall is removed, and characterizes its composition in terms of protein, amino acids, carbohydrates, vitamins and minerals.[48] The industry distinction between a yeast extract (cell walls removed) and a yeast autolysate (cell walls retained) is compositionally meaningful and analytically verifiable.

6.2 Conventional Specification Parameters

Table 3 lists the parameters by which yeast extracts and yeast-extract spreads are conventionally specified and released, with the methods normally applied and the aspect of the product each parameter is intended to control. The list is compiled from the industrial and academic literature reviewed above and from the regulatory texts.[4,6,32,43]

Table 3. Conventional quality-control parameters for yeast extracts and yeast-extract spreads.

Parameter	Usual method	What it controls; comment
Dry matter / moisture; water activity	Oven or Karl Fischer; aw meter	Concentration end-point; microbial stability of the paste
Sodium chloride; sodium; potassium	Potentiometric chloride titration; flame photometry / ICP	Salt dosing; label sodium; KCl substitution
Total nitrogen (Kjeldahl or Dumas) × 6.25	Combustion or digestion	"Protein" for labeling; includes non-protein nitrogen (nucleotides, ammonium, choline)
α-Amino nitrogen; free amino nitrogen; AN/TN ratio	Formol titration, ninhydrin, OPA; amino acid analysis	Degree of hydrolysis; process end-point; taste intensity
Free glutamic acid	Enzymatic (glutamate dehydrogenase); HPLC after derivatization	Umami strength; labeling claims
5′-Ribonucleotides (IMP, GMP, AMP); total nucleotides	Ion-pair or anion-exchange HPLC with UV detection	Flavor-enhancing activity of nucleotide-enriched grades; process verification
pH; titratable acidity	Potentiometric	Process consistency; shelf stability
Color	Absorbance at 420–550 nm of dilutions; CIE L*a*b*	Thermal history; caramel dosing
Ash; individual minerals; heavy metals	Gravimetric; ICP-OES / ICP-MS	Label minerals; regulatory limits (21 CFR 184.1983)
B vitamins	HPLC (fluorescence or UV); microbiological assay; LC-MS/MS for folate vitamers	Fortification to label; native content
Microbiology	Plate counts; pathogen screens	Regulatory limits; spread products are effectively sterile
Biogenic amines (tyramine, histamine, putrescine, cadaverine)	HPLC with derivatization; LC-MS/MS	Raw-material hygiene; MAOI interaction risk
3-MCPD, 1,3-DCP (HVP-containing products only)	GC-MS after derivatization	Acid-hydrolysis process contaminants
Gluten / cereal allergens	ELISA; LC-MS/MS peptide markers	Allergen labeling; brewer's-yeast origin
Sensory	Trained panel; reference lot comparison	Overall conformity; bitterness; off-notes

6.3 Limitations of the Conventional Framework

Each parameter in Table 3 measures one attribute, and most measure it as a sum: total nitrogen sums every nitrogen-containing species, free amino nitrogen sums every primary amine, and even the individual amino acid and nucleotide assays require separate sample preparations and calibrations. None of them describes the product as a whole, and a batch that meets every specification can still differ materially in the ratio of free to peptide-bound amino acids, in the nucleotide speciation, in the residual trehalose or dextrin, in the organic acid pattern or in the choline fraction. The NMR-based lot-to-lot study of hydrolysates for cell culture concluded exactly this: significant differences between lots existed, but they were driven by a relatively small fraction of the compounds, which a targeted specification would have missed unless it happened to include them.[7] A whole-spectrum method that observes every soluble constituent in one measurement, that requires no calibration for identification and only an internal standard for quantitation, and that yields a fingerprint amenable to statistical process control, is the natural complement to the targeted framework, and the case for it is developed in the remainder of this paper.

7. NMR Spectroscopy Applied to Yeast Extracts: Principles and Methods

7.1 Literature Precedent

Direct NMR studies of yeast extracts as food or media ingredients are few, but they are instructive. The earliest, a ^{1}H and ^{31}P study of Luria–Bertani broth, found that the yeast-extract component contributed the high level of 2′,3′-cyclic nucleotides that dominate the ^{31}P spectrum of the medium, and traced them to ribonuclease action during autolysis.[34] A comprehensive NMR metabolomic assessment of nine hydrolysate products — four plant-derived and five yeast-derived, each in two to four lots — identified 90 unique metabolites, found only 15 common to all products, quantified the metabolite mass fraction at 14 percent in soy hydrolysates and up to 43 percent in yeast extract, noted the prominence of nucleosides in two of the yeast products, and showed that lot-to-lot differences, though significant, were carried by a small subset of compounds.[7] The same group had earlier developed a Plackett–Burman approach for estimating the quantification error of individual metabolites in convoluted one-dimensional ^{1}H spectra of complex media, an issue directly relevant to spread spectra in which the 3.5 to 4.0 ppm region is a near-continuous envelope.[60] Optimized protocols for the ^{1}H NMR metabolic profiling of *S. cerevisiae* cells — extraction, buffering and referencing — provide the assignment base for the intracellular metabolites that survive into an autolysate.[61] The classic *in vivo* ^{31}P and ^{13}C studies of intact yeast, which resolved orthophosphate, sugar phosphates, nucleotide phosphates and polyphosphate and followed the fate of ^{13}C-labeled glucose through glycolysis, trehalose and glycogen, define the phosphorus and carbohydrate species that the extract inherits.[39,56] Beyond these, the general food-NMR literature — reviews of NMR in food science and of NMR-based multiparametric quality control of juices — supplies the operational model for a fingerprint-plus-quantitation scheme.[10,62]

Two related bodies of NMR work deserve mention because they bear on the interpretation of the spreads. The first is the NMR metabolomics of soy sauce, a fermented product whose free amino acid, organic acid and sugar inventory overlaps substantially with that of a yeast extract and whose authenticity problem — the substitution of fermented product by acid-hydrolyzed vegetable protein — is the analog of the substitution discussed in Section 10; ^{1}H NMR profiling of soy sauces has been reported, and levulinic acid, formed by acid degradation of carbohydrate, is the accepted chromatographic marker of the acid-hydrolyzed product.[63–65] The second is the extensive NMR literature on carbohydrates and glucans, which provides the assignments for trehalose, sucrose, maltodextrin, glycogen and mannan that are needed to read the anomeric region of the spread spectra.[53–55]

7.2 Sample Preparation

The spreads are water-based pastes of 70 to 80 percent solids and dissolve readily in D_2O. For qualitative profiling a loading of 50 to 100 mg of paste per milliliter of D_2O is convenient; the resulting solution is dark but transparent, and a brief centrifugation removes the small amount of insoluble cell-wall and cereal material. Three aspects of the preparation deserve attention. The first is pH. Many of the diagnostic signals — succinate, acetate, lactate, citrate, glutamate γ-CH_2, aspartate β-CH_2, the histidine and nucleotide ring protons and the α-protons of every amino acid — shift by tenths of a part per million between pH 5 and pH 8, and the pastes themselves are mildly acidic; the pH dependence of metabolite shifts is documented in the public shift compilations, and the practical remedy is a phosphate buffer of 0.1 to 0.2 M at pH 7.0 ± 0.05 in the D_2O, with the pH of the final solution recorded.[61] The second is referencing. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) or 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) is added as the ^1H reference at 0.00 ppm and doubles as the internal quantitation standard; for ^{13}C the DSS scale differs from the tetramethylsilane scale by about 2 ppm, and published ^{13}C shift compilations for sugars and amino acids must be read with that offset in mind.[52] TSP binds to proteins and peptides, so that in a peptide-rich autolysate a non-binding standard (DSS in a capillary, or an electronic reference) is preferable for quantitation. The third is ionic strength: at 100 mg of paste per milliliter the salt contribution alone is roughly 0.15 to 0.19 M sodium chloride, and the conductivity lowers probe quality factor, lengthens the 90° pulse and reduces sensitivity, effects that are well characterized and that argue for consistent loading from sample to sample and for calibration of the pulse width on a representative sample.[41] Iron-fortified products may show shortened relaxation times and broadened lines; if this is observed, chelation with a small excess of EDTA, or a check of the linewidth of the TSP reference against a non-fortified product, is advisable.

7.3 One-Dimensional ^1H NMR

The residual HOD signal at 4.79 ppm (298 K) is suppressed by presaturation, by a NOESY-presaturation sequence or by excitation sculpting, the last being preferred where signals close to the water resonance (the anomeric protons of β-glucose and of β-linked residues at 4.5 to 4.7 ppm, and the α-protons of threonine and of peptides) must be preserved.[66] For quantitative work a 30° or calibrated 90° pulse, a relaxation delay of at least five times the longest relevant T_1 (which for the methyl singlets of acetate and choline and for formate can exceed 5 s), a spectral width of at least 16 ppm and an acquisition time of 3 to 4 s are required; 64 to 128 transients at 300 MHz give adequate signal-to-noise for the major constituents at the loading suggested above.[8,9] Processing should include mild exponential apodization (0.3 Hz), careful zero- and first-order phasing, and a baseline correction that does not erode the broad components. Signal assignment draws on public databases of metabolite spectra and on the compilations of amino acid random-coil shifts for the peptide fraction.[52,67,68]

7.4 ^{13}C NMR and DEPT Editing

Proton-decoupled ^{13}C NMR is the natural complement to ^1H NMR for a yeast extract because its 200 ppm dispersion separates the carboxyl, aromatic, anomeric, carbinol, α-carbon, methylene and methyl regions cleanly, and because the quaternary carbons that are silent in ^1H NMR — carboxylates, amides, the guanidino carbon of arginine, the C-4 of tyrosine, the purine and pyrimidine ring carbons and the C-2′ of the fructose residue of sucrose — are directly observed. At natural abundance and at 7.05 T the technique is not sensitive, and a 100 mg/mL solution requires several thousand transients; the resulting spectrum is not quantitative unless acquired with inverse-gated decoupling and long relaxation delays, but it is fully usable for identification and for ratio comparisons within a class. DEPT-135 and DEPT-90 editing (or, equivalently, the multiplicity-edited HSQC discussed below) separates CH_3, CH_2 and CH carbons and removes quaternary carbons, which converts the crowded 15 to 30 ppm and 60 to 80 ppm regions into readily assignable sub-spectra: the CH_3 sub-spectrum contains the alanine, threonine, valine, leucine, isoleucine, lactate, acetate and choline N-methyl carbons; the CH_2 sub-spectrum contains the sugar C-6

carbons, the lysine, arginine, leucine and glutamate side-chain carbons and succinate; and the CH sub-spectrum contains the anomeric and ring carbons of the sugars, the α-carbons of the amino acids and the aromatic CH carbons.[52,53]

7.5 Two-Dimensional Methods

Gradient-selected ^{1}H–^{13}C HSQC resolves the one-bond correlations and, in its multiplicity-edited form, encodes CH/CH_3 and CH_2 in opposite phase, so that a single experiment provides both the connectivity and the DEPT information; HSQC cross-peak volumes can be calibrated to give molar concentrations in complex mixtures, an approach that circumvents the ^{1}H overlap that limits one-dimensional quantitation in the 3.5 to 4.0 ppm region of a spread spectrum.[69] Gradient-selected HMBC reveals two- and three-bond correlations and is the experiment that ties methyl and methylene signals to their carboxyl, carbinol and ring carbons — the alanine CH_3 to its carboxylate and α-carbon, the glutamate γ-CH_2 to its two carboxylates, the choline N-methyl to the N-methylene, the trehalose H-1 across the glycosidic oxygen to C-1′ of the second ring, and the sucrose H-1 to C-2′ of the fructose residue — and that distinguishes free amino acids from their peptide-bound forms through the amide versus carboxylate carbonyl shift. Homonuclear 2D J-resolved spectroscopy separates chemical shift from scalar coupling and is a standard tool of metabolomics for confirming multiplicities in crowded regions, with the caveat that strongly coupled spin systems, common at 300 MHz, produce additional off-diagonal responses.[70] TOCSY connects the spin systems of the individual amino acids and sugars and is invaluable for assigning the branched-chain amino acids and the sugar ring protons. Diffusion-ordered spectroscopy (DOSY) separates signals by molecular size and is the experiment of choice for confirming that a 5.4 ppm anomeric envelope belongs to a polymeric dextrin or glycogen rather than to a small oligosaccharide, and for distinguishing peptide-bound from free amino acid signals.[71]

7.6 ^{31}P NMR

Phosphorus-31 NMR, at 100 percent natural abundance and with a chemical shift range that resolves phosphomonoesters, phosphodiesters, orthophosphate, pyrophosphate, polyphosphate and the 2′,3′-cyclic phosphates, offers a direct and uncrowded view of the nucleotide chemistry of Section 4.3 and of the phospholipid-derived and inorganic phosphorus of Section 5.5. The cyclic nucleotides resonate at 20 to 21 ppm, well clear of the 5′-, 3′- and 2′-monoester region near 3 to 5 ppm (pH-dependent), glycerophosphocholine near 0 ppm, orthophosphate near 2 to 3 ppm, pyrophosphate near −5 to −7 ppm and the internal residues of polyphosphate near −20 ppm.[34,39] Because the spreads are phosphorus-rich (declared phosphorus in yeast extracts reaches several grams per 100 g), a ^{31}P spectrum of adequate quality is obtained in a few hundred transients, and it constitutes the most direct test of whether an extract was made by the endogenous-nuclease route (cyclic and 3′-nucleotides) or the 5′-phosphodiesterase route (5′-nucleotides), and of whether disodium 5′-inosinate and guanylate have been added.

7.7 Benchtop NMR

Permanent-magnet benchtop spectrometers operating at 60 to 80 MHz have matured into practical tools for chemical and biochemical analysis, and their limitations — fivefold lower dispersion than a 300 MHz instrument and a sensitivity lower by more than an order of magnitude (the B₀^3/2 dependence alone gives a factor of about eleven) — are well documented.[72] For yeast-extract spreads the consequences are predictable. The isolated singlets and simple doublets that carry most of the quality information — formate, the nucleobase H-8 and H-2 singlets, the trehalose and sucrose anomeric doublets, choline, glycine, succinate, acetate, the alanine doublet and, in aggregate, the branched-chain methyl region — remain observable at 60 MHz, and a whole-spectrum fingerprint suitable for principal-component monitoring is obtainable in a few minutes at a loading of 100 mg per milliliter. The 3.5 to 4.0 ppm envelope, the 2.0 to 2.5 ppm glutamate/glutamine/proline region and the 0.9 to 1.1 ppm methyl region collapse into unresolved bands, so that individual amino acid quantitation, other than alanine, is not realistic. A dual-tier scheme —

benchtop fingerprinting for routine batch-to-batch consistency and high-field qNMR for full quantitation and for investigation of out-of-trend batches — is the pragmatic configuration for a producer, and it is the configuration that the PNA laboratory, with 300 MHz and 60 MHz instruments in the same room, is equipped to demonstrate.

7.8 Quantitative NMR Practice

Quantitative ^{1}H NMR rests on the proportionality between integrated signal area and the number of nuclei contributing to it, and its accuracy depends on complete relaxation between transients, uniform excitation across the spectral width, correct phasing and baseline, and integration of signals that are free of overlap or whose overlap is resolved by deconvolution or by a 2D method.[8,9] For a spread, the analytes that are quantifiable from a one-dimensional spectrum at 300 MHz with an internal standard are those with isolated signals: formate (8.46 s), the nucleobase H-8 protons, the trehalose H-1 doublet, the sucrose H-1 doublet, the α-glucan anomeric envelope (as total α-(1→4)-linked glucose), the α- and β-glucose anomeric doublets, the tyrosine and phenylalanine aromatic signals, choline (3.20 s), glycine (3.56 s), succinate (2.41 s), acetate (1.92 s), the alanine doublet (1.48 d), the lactate doublet (1.33 d, provided threonine is deconvoluted), and the aggregate branched-chain methyl region as a proxy for valine plus leucine plus isoleucine. Glutamate, the most important taste analyte, is best quantified from its γ-CH_2 multiplet at 2.34 ppm with deconvolution from glutamine (2.45) and pyroglutamate, or from an HSQC calibration. Reporting on a dry-matter basis requires a separate moisture determination. The uncertainty budget for such measurements in complex matrices has been treated formally, and a 2 to 5 percent relative standard deviation is achievable for the well-resolved analytes.[60]

8. The PNA Data Set: Five Commercial Spreads by Multinuclear NMR at 300 MHz

8.1 Samples and Experiments

Five retail products were examined at Process NMR Associates on a Varian Mercury VX 300 MHz spectrometer (7.05 T; instrument designation JCE-PNA-MVX300 in the figure headers) by dissolution in D_2O: Marmite (Unilever, lot L0308A1795), Marmite XO (Unilever, lot L2276A1795), Vegemite (Bega, lot K15SEP21B), Sanitarium Marmite (New Zealand) and Promite (Mars Food, Australia). One-dimensional ^{1}H spectra were acquired for all five; proton-decoupled ^{13}C spectra were acquired for Marmite XO, Vegemite, the New Zealand Marmite and Promite; DEPT-edited ^{13}C sub-spectra (CH_3, CH_2, CH and the composite CH_n) for Marmite XO, Vegemite and the New Zealand Marmite; gradient-selected multiplicity-edited ^{1}H–^{13}C HSQC spectra for all five; gradient-selected ^{1}H–^{13}C HMBC spectra for Marmite XO, Vegemite and the New Zealand Marmite; and a homonuclear 2D J-resolved spectrum for Marmite XO. The experiment inventory is summarized in Table 4, and the figures reproduce the shift-corrected data set supplied by the author in September 2026, which supersedes the preliminary figure set used for version 1.0 of this document. For version 1.2 the author supplied the processed data themselves, as five PNA NMR Processor workspaces that carry the MestReNova documents of the five products, and the two-dimensional spectra were assigned from those numerical data (Sections 8.10 to 8.15).

Chemical shifts in ^{1}H are referenced to the trimethylsilyl standard at 0.00 ppm in every one-dimensional spectrum; the ^{1}H axes of the two-dimensional spectra depart from that reference by up to 0.09 ppm and were aligned to the one-dimensional spectra for the analysis of Sections 8.10 to 8.15. The ^{13}C axes were referenced indirectly and are not on a single common scale across the data set. In version 1.1 the scales were judged from the figures, and the Marmite XO ^{13}C spectrum and the gHSQC F1 axes were taken to lie on the tetramethylsilane (TMS) scale; the numerical data examined for version 1.2 (Section 8.10 and Table 8) show the offsets to be larger and less uniform than that. Relative to the DSS scale used by the public metabolite databases, on which the alanine methyl carbon lies at 18.83 ppm, the Marmite XO ^{13}C spectrum

reads 3.11 ppm low (alanine methyl at 15.75 ppm), the New Zealand Marmite and Promite ^{13}C spectra 2.91 and 2.52 ppm low (sucrose C-2′ at 103.45 and 103.83 ppm), the F1 axes of the five gHSQC spectra 2.40 to 2.55 ppm low and those of the three gHMBC spectra 2.85 to 5.05 ppm low, whereas the Vegemite ^{13}C spectrum and the DEPT sub-spectra lie within 0.25 ppm of the DSS scale. In Sections 8.3 to 8.8, ^{13}C positions are quoted as they were read from the figure concerned for version 1.1 and are approximate; in Sections 8.10 to 8.15 every ^{13}C position has been measured from the numerical data and corrected to the DSS scale. Table 5 gives literature values on the TMS scale, which are about 2 ppm lower than the DSS-scale values. Two caption slips in the source figures are noted for the record: the DEPT panels are captioned "in $CDCl_3$", although the observed carbons are those of the water-soluble constituents and the spectra are evidently of the D_2O solutions, and the ^{13}C spectrum of Vegemite is captioned "Vegemite XO". Both are taken to be template carry-overs, and the parameter sets of the DEPT experiments, and of two of the gHMBC experiments, likewise name solvents other than D_2O. The interpretation of Sections 8.3 to 8.9 is qualitative and was made from the figures; no integrals were available.

Table 4. Samples and NMR experiments in the PNA data set (Varian Mercury VX 300, D_2O; shift-corrected figure set of September 2026).

Product (lot)	Experiments	Figure(s) in this document
Marmite, Unilever UK (L0308A1795)	^{1}H; ^{1}H–^{13}C gHSQC (multiplicity-edited)	Figures 1 and 2
Marmite XO, Unilever UK (L2276A1795)	^{1}H; $^{13}C\{^{1}H\}$; DEPT ($CH_3/CH_2/CH/CH_n$); gHSQC; gHMBC; 2D J-resolved	Figures 3 to 8
Vegemite, Bega Australia (K15SEP21B)	^{1}H; $^{13}C\{^{1}H\}$; DEPT; gHSQC; gHMBC	Figures 9 to 13
Sanitarium Marmite, New Zealand	^{1}H; $^{13}C\{^{1}H\}$; DEPT; gHSQC; gHMBC	Figures 14 to 18
Promite, Mars Food Australia	^{1}H; $^{13}C\{^{1}H\}$; gHSQC	Figures 19 to 21

8.2 A Region-by-Region Assignment Framework

The ^{1}H spectrum of a yeast-extract spread in D_2O divides naturally into six regions, and Table 5 lists the constituents expected in each together with their characteristic ^{1}H and ^{13}C positions. The table was assembled from the public metabolite databases, from the carbohydrate assignment literature and from the random-coil shift compilations, and it is offered as the working key to Figures 1 to 21; assignments that are marked tentative are those that rest on chemical shift alone and that have not been confirmed by a 2D correlation or by spiking in the present data set.[52–54,67]

Table 5. Working assignment key for ^{1}H and ^{13}C NMR spectra of yeast-extract spreads in D_2O. ^{1}H shifts are relative to TSP/DSS at 0.00 ppm; ^{13}C shifts are given on the TMS scale (DSS-scale values, as tabulated in the Human Metabolome Database and used in Sections 8.10 to 8.15, are approximately 2 ppm higher; the F1 axes of the gHSQC figures read 2.40 to 2.55 ppm below the DSS scale, Table 8). Shifts of ionizable species are pH-dependent; values are for neutral pH unless stated. The positions observed in the two-dimensional spectra are given, on the DSS scale, in Table 9, which supersedes this key where the two differ — notably for the histidine H-ε1 proton, which lies at 8.60 ppm at the apparent pH of these solutions.

Region	Constituent and signal	^{1}H δ (ppm), multiplicity	^{13}C δ (ppm, TMS scale) and notes
9.5–8.0 (aromatic/heteroaromatic)	Formate	8.46 s	171; marker of sugar degradation / acid hydrolysis
	Niacin (nicotinate) H-2, H-6, H-4, H-5	8.94 s, 8.62 d, 8.28 d, 7.53 dd	~150/149 (C-2/C-6), ~137 (C-4), ~124 (C-5); fortificant
	5′-AMP / adenosine H-8, H-2	8.55–8.62 s, 8.25–8.27 s	~141 (C-8), ~153 (C-2); C-4/C-5/C-6 ~149/119/156

Region	Constituent and signal	^{1}H δ (ppm), multiplicity	^{13}C δ (ppm, TMS scale) and notes
	5′-IMP / inosine / hypoxanthine H-8, H-2	8.3–8.6 s, 8.2 s	~141/147; hypoxanthine 8.19/8.21
	5′-GMP / guanosine H-8	8.15–8.20 s	~138 (C-8)
	Uridine / UMP H-6; cytidine / CMP H-6	7.87–8.10 d	~142 (C-6); C-2 ~152, C-4 ~166 (uracil)
	Histidine H-2 (ring)	7.8–8.0 s (pH-dependent)	~136
8.0–6.5 (aromatic)	Phenylalanine ring H	7.32–7.43 m	130.0, 129.5, 128.4; C-1 ~136
	Tyrosine H-2/6, H-3/5 (also tyramine)	7.19 d, 6.90 d	131.3, 116.5; C-4 ~155, C-1 ~128
	Tryptophan ring H	7.73 d, 7.54 d, 7.28 t, 7.20 t, 7.32 s	~112–137
	Histidine H-4	7.05–7.10 s	~118
6.5–4.5 (anomeric/olefinic)	Purine nucleotide H-1′ (AMP, IMP)	6.10–6.15 d	~87–88 (C-1′)
	GMP H-1′; uridine/UMP H-5 and H-1′; cytidine H-5	5.90–6.05 d	~87–89 (C-1′); uracil C-5 ~103
	Sucrose glucosyl H-1	5.41 d (J ≈ 3.9 Hz)	92.9 (C-1); 104.5 (C-2′, quaternary)
	α-(1→4)-Glucan (dextrin, maltodextrin, glycogen) H-1	5.35–5.42 br	100.4 (C-1); linked C-4 ~77.5–78
	Unsaturated fatty acyl CH=CH (lipid, free fatty acid)	5.30–5.38 m	~128–130 (olefinic CH); tentative
	Maltose/maltotriose non-reducing H-1′	5.40 d	~100.5
	α-Glucose (free or reducing end) H-1	5.23 d (J ≈ 3.7 Hz)	92.9 (α) / 96.7 (β, H-1 4.65 d, J ≈ 8 Hz)
	Trehalose H-1 (both rings)	5.19 d (J ≈ 3.8 Hz)	94.0 (C-1); 73.3, 72.9, 71.9, 70.5 (ring); 61.4 (C-6)
	α-(1→6)-Glucan branch H-1; mannan H-1	4.95–5.05 br	~98.5–101
	β-Glucan / β-linked residues H-1	4.5–4.8 d	~103
4.5–3.0 (carbinol / α-CH / N-CH)	Sucrose H-3′, H-4′, H-1′ (s), ring H	4.22 d, 4.05 t, 3.67 s, 3.4–3.9	82.2 (C-5′), 77.3 (C-3′), 74.9 (C-4′); 63.2, 62.3, 61.0 (CH_2)
	Amino acid α-CH (Gly 3.56 s; Ala 3.78 q; Glu 3.75; Leu 3.73; Val 3.61 d; Lys 3.76; Thr 3.58/4.25)	3.5–4.3	Cα 42.3 (Gly), 51.5 (Ala), 55.4 (Glu), 61.2 (Val), 54.2 (Leu), 55.2 (Lys); Thr Cβ 66.7
	Choline $N(CH_3)_3$; glycerophosphocholine; (betaine 3.27 s + 3.90 s)	3.20 s; 3.23 s	54.5 (N-CH_3); N-CH_2 68.0; CH_2OH 56.3
	Lysine ε-CH_2; arginine δ-CH_2; tyrosine/phenylalanine β-CH_2; GABA γ-CH_2	3.02 t; 3.24 t; 3.0–3.3; 3.01 t	39.7; 41.3; 36–37; 40.0
	Glycerol	3.56, 3.65, 3.78	63.0, 72.6
3.0–1.8 (methylene)	Aspartate / asparagine β-CH_2	2.68–2.95 dd	37.4 / 35.3
	Citrate CH_2 (AB)	2.53, 2.66 d (J ≈ 15 Hz)	46.3; 76.1 (C-3, quaternary)
	Glutamine γ-CH_2 / glutamate γ-CH_2 / β-CH_2	2.45 m / 2.34 m / 2.05–2.13 m	31.7 / 34.2, 27.7; Cδ ~180–182
	Succinate	2.41 s (2.45–2.6 at pH 4–5)	34.8; 182.5
	Pyroglutamate; proline; methionine S-CH_3	2.0–2.5 m; 2.0/2.35 m; 2.13 s	—; 24.5/29.8; 14.7

Region	Constituent and signal	^{1}H δ (ppm), multiplicity	^{13}C δ (ppm, TMS scale) and notes
	Levulinate CH_3, CH_2 (acetone CH_3 2.22 s / 30.9 also possible)	2.22 s, 2.42 t, 2.75 t	~29.6 (CH_3); ~38 and ~30 (CH_2); ~216 (C=O); HVP marker (tentative)
	Acetate	1.92 s	24.0; 182.0
1.8–0.8 (methyl)	Leucine, lysine, arginine CH_2; isoleucine γ-CH_2	1.65–1.75 m; 1.25/1.47 m	~25–31
	Alanine CH_3	1.48 d (J ≈ 7.2 Hz)	17.0
	Lactate CH_3 / threonine CH_3	1.33 d / 1.32 d	20.9 (CH 69.3; COO^- 183) / 20.4
	Ethanol CH_3	1.18 t	17.6; CH_2 58.2 (3.65 q)
	Valine, leucine, isoleucine CH_3	0.94–1.05 d and t	17.5–22.7; Ile δ-CH_3 11.8

8.3 Marmite (Unilever, United Kingdom)

The ^{1}H spectrum of Marmite (Figure 1) is the spectrum of a highly hydrolyzed yeast autolysate. The tallest signal in the spectrum is the alanine methyl doublet at 1.48 ppm, followed by the branched-chain methyl cluster at 0.94 to 1.05 ppm, the crowded carbinol and α-CH envelope between 3.5 and 4.0 ppm, and three prominent sharp singlets: at 3.24 ppm (a trimethylammonium group, assigned to choline and glycerophosphocholine and corroborated in Section 8.4), at 2.47 ppm (succinate, whose position is shifted downfield from its neutral-pH value of 2.41 ppm by the mildly acidic sample and is therefore also a crude pH indicator) and at 1.96 ppm (acetate). The lactate/threonine doublet at 1.33 ppm, the lysine ε-methylene triplet at 3.02 ppm, the glutamate γ-methylene multiplet at 2.35 ppm with the glutamine and pyroglutamate multiplets around it, the glycine singlet at 3.56 ppm and a singlet at 2.14 ppm (methionine S-methyl and glutamine β-CH_2 region) complete the aliphatic inventory.

The downfield region is unusually rich for a food spectrum. Phenylalanine (7.32 to 7.43 ppm) and tyrosine (7.18 and 6.88 ppm doublets) are strong, tryptophan is discernible at 7.5 to 7.75 ppm, and between 7.85 and 8.65 ppm a series of sharp singlets and doublets at 8.60, 8.35, 8.22, 8.02, 7.88 and 7.85 ppm, together with the doublets between 5.9 and 6.15 ppm, record, in the main, the purine and pyrimidine nucleosides and bases liberated from yeast RNA. As read from the figure for version 1.1, the 8.60/8.22 pair was attributed to the adenine ring of 5′-AMP or adenosine, the 8.35/8.22 pair to the hypoxanthine ring of inosine or 5′-IMP, the 8.2 ppm singlet to the guanine H-8, and the 7.85 to 8.02 ppm doublets to the H-6 protons of uridine, cytidine and their nucleotides, whose H-5 and H-1′ doublets lie at 5.9 to 6.05 ppm. The long-range correlations examined for version 1.2 (Section 8.12) revise that reading in two respects: the 8.60 ppm singlet is the H-ε1 proton of free histidine in its imidazolium form and not a purine H-8, the adenine-ring protons being the pair at 8.34 and 8.21 ppm; and uridine is established whereas cytidine is not found. A weak singlet at 8.94 ppm and the accompanying signals at 8.62 and 8.28 ppm are consistent with the fortificant niacin; the sharp singlet at 8.47 ppm is formate.

The anomeric region distinguishes this product. A broad signal centered at 5.40 ppm correlates in the HSQC (Figure 2) to a carbon at 100 ppm, and a weaker companion cross-peak at δH 5.0/δC 98.5 lies where the anomeric carbon of an α-(1→6)-linked branch residue is expected; together they are the signature of an α-(1→4)-linked glucan carrying α-(1→6) branch points, which in a brewer's-yeast product may be the residual glycogen of the yeast, the dextrins of the wort carried in with the yeast slurry, or both.[54,55] A third anomeric cross-peak near δH 5.1/δC 101.5 to 102 is in the range of mannan and of non-reducing α-(1→4) residues adjacent to a branch. Beside these stands a sharp doublet at 5.20 ppm with an HSQC correlation at 93.5 ppm, which is assigned to trehalose (C-1 94.0 ppm on the TMS scale), and small signals near 5.22 and 4.65 ppm from the α and β anomers of free glucose. In the HSQC the nucleoside H-1′ protons at 5.9 to 6.05 ppm correlate to carbons at 88 to 89 ppm, the tyrosine H-3/5 to 116 ppm, the tyrosine H-2/6 to 130.5 ppm

and the phenylalanine ring protons to 129 ppm; the choline N-methyl cross-peak lies at δH 3.24/δC 54.5, the lysine ε-methylene (negative phase) at 3.02/40, the glycine methylene (negative) at 3.56/42.5, succinate at 2.47/34.5, acetate at 1.96/24 and the alanine methyl at 1.48/17.0, each within 0.5 ppm of its TMS-scale literature value. The negative (CH_2) cross-peaks between δH 1.5 to 3.1 and δC 25 to 45 map the lysine, arginine, leucine, glutamate, aspartate and succinate methylenes, and the positive cross-peaks between δH 0.9 to 1.5 and δC 15 to 23 map the methyl carbons of alanine, threonine, valine, leucine, isoleucine and lactate.

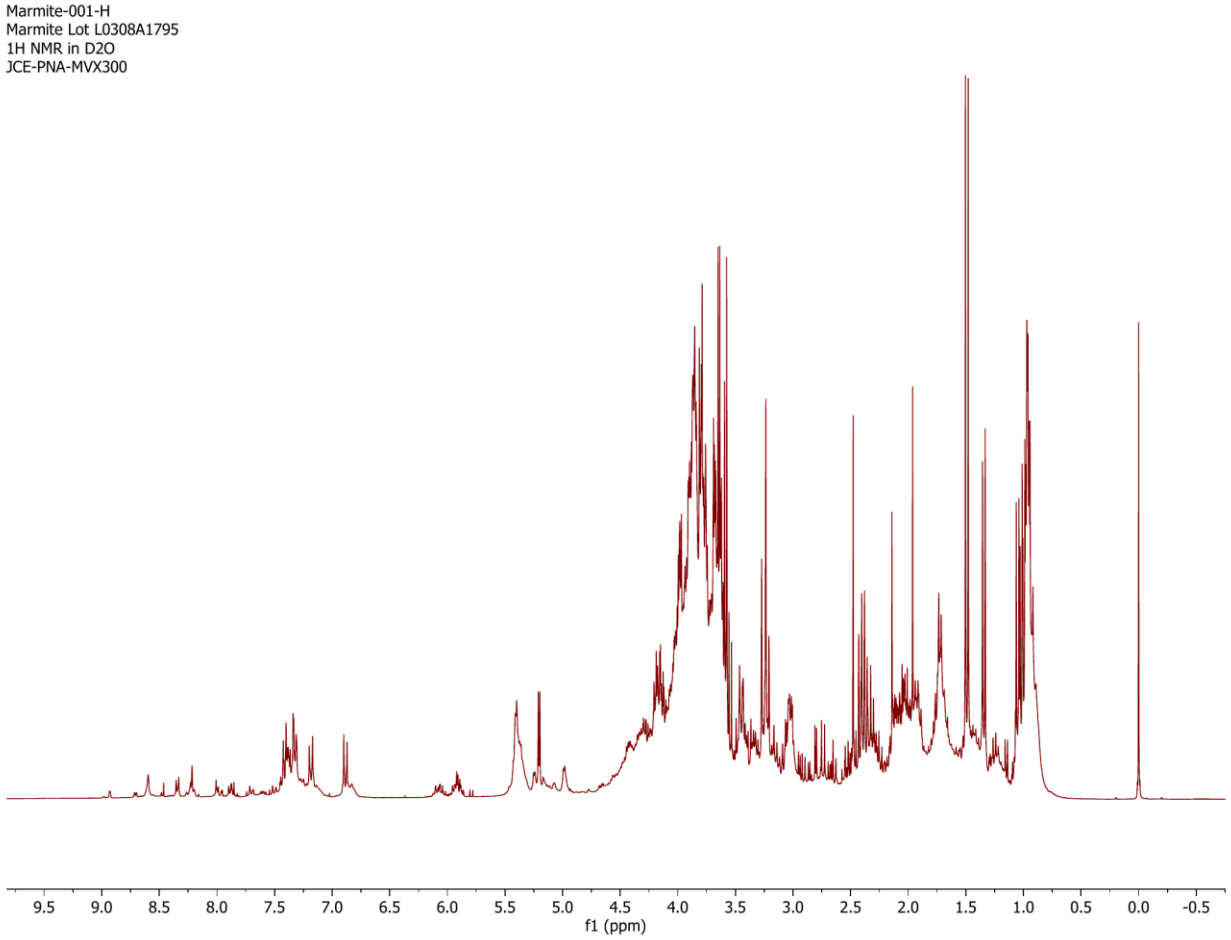


Figure 1. ^1H NMR spectrum (300 MHz, D_2O) of Marmite, lot L0308A1795. Process NMR Associates, JCE-PNA-MVX300.

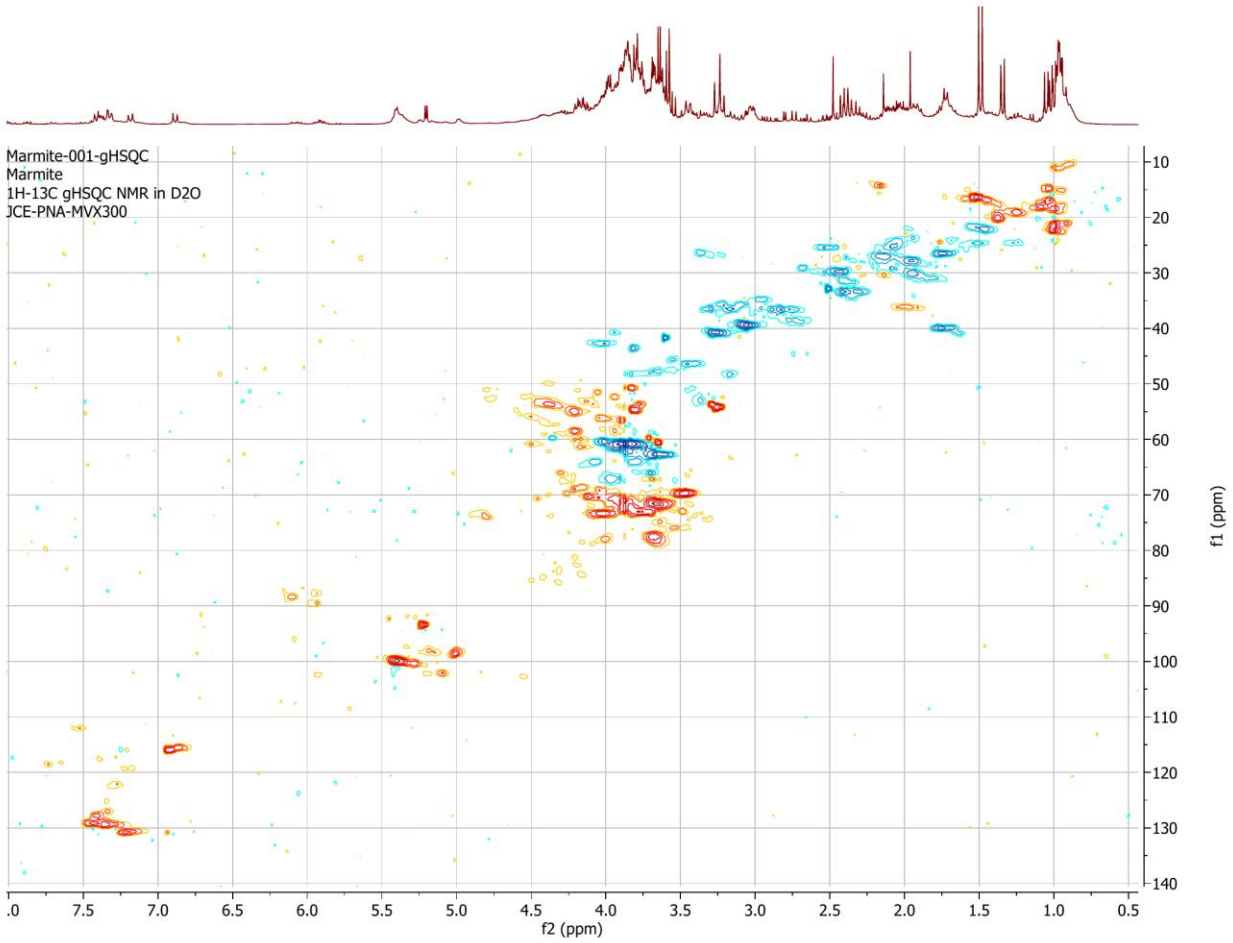


Figure 2. Multiplicity-edited $^{1}H–^{13}C$ gHSQC spectrum (300 MHz, D_2O) of Marmite. CH and CH_3 correlations are drawn in red/orange and CH_2 correlations in blue; the F1 axis reads 2.55 ppm below the DSS scale (Table 8).

8.4 Marmite XO

The ^{1}H spectrum of Marmite XO (Figure 3) reproduces every feature of the standard product, which is as expected for a product described by its manufacturer as the same recipe matured for four times as long, but with two qualitative differences that are consistent with a longer digestion. The trehalose doublet at 5.20 ppm is markedly weaker relative to the α-glucan envelope at 5.40 ppm and to the amino acid signals than in Figure 1, as would follow from more complete trehalase action, and the purine region between 8.0 and 8.65 ppm is relatively more intense, consistent with further release of nucleotides and bases. Relative intensities read from spectra that are scaled to their tallest peak must be treated with caution, and the comparison is offered only as a hypothesis for quantitative testing; if it is confirmed by integration against an internal standard, the trehalose-to-α-glucan ratio and the nucleobase-to-aromatic-amino-acid ratio would be candidate maturation indices for the XO product.

The proton-decoupled ^{13}C spectrum (Figure 4) confirms and extends the ^{1}H assignments. The positions quoted in this paragraph are those estimated from the figure for version 1.1, on the assumption that its axis lay on the TMS scale, and are approximate; the numerical data show the axis to read 3.11 ppm below the DSS scale, and the positions measured from the data are given on the DSS scale in Section 8.11 and Table 9. The carboxyl and carbonyl region contains the glutamate γ-carboxylate, succinate and acetate carboxylates at 180 to 182 ppm, the free amino acid α-carboxylates at 176 to 178 ppm and the peptide and amide carbonyls at 172 to 175 ppm. The arginine guanidino carbon and the C-2, C-4 and C-6 carbons of

the purine and pyrimidine rings lie at 152 to 157 ppm, the nucleobase C-8 carbons near 141 ppm, the aromatic carbons of phenylalanine and tyrosine at 128 to 135 ppm and the tyrosine C-3/5 at 116 ppm. The anomeric region shows the α-glucan C-1 at 100 ppm with companions at 102 and 98.5 ppm, a weak signal at 92.5 ppm (reducing-end or trehalose C-1) and the nucleoside C-1′ near 88 ppm; the linked C-4 of the glucan appears near 78 ppm. The sugar ring carbons cluster at 70 to 74 ppm, the choline N-methylene at 68 ppm, the threonine β-carbon at 66.5 ppm and, as read for version 1.1, glycerol at 63 ppm and the glucan/trehalose C-6 at 61.8 ppm, the tallest signal in the spectrum. The numerical data correct that last attribution: the tallest line lies at 62.07 ppm on the axis of the figure (65.18 ppm on the DSS scale) and is the CH_2 carbon of glycerol, the C-6 carbons lying about 2 ppm upfield of it (Section 8.11). The α-carbons occupy 51 to 62 ppm (alanine 51.5, leucine and lysine 54 to 55, glutamate 55.6, serine and proline 58 to 62) with the choline N-methyl at 54.5 ppm among them; proline C-δ is at 46 ppm, glycine at 42.4 ppm, the lysine ε-carbon and leucine β-carbon at 40 ppm, aspartate β at 37 ppm, succinate at 34.8 ppm and glutamate γ at 34.2 ppm, followed by the glutamate β, lysine δ and proline β carbons at 27 to 30 ppm, leucine γ and acetate at 24 to 25 ppm, the leucine methyls and lysine γ at 22.3 to 22.7 ppm, threonine methyl at 20.5 ppm, valine methyls at 18.6 and 17.5 ppm, the alanine methyl (the tallest methyl signal, in agreement with the ^1H spectrum; it lies at 15.75 ppm on the axis of the figure, and not at 17.0 ppm as was stated in version 1.1), the isoleucine methyls at 15.4 and 11.8 ppm. The DEPT sub-spectra (Figure 5) sort these into their multiplicity classes: the CH_3 trace carries the amino acid and lactate methyls together with methyl carbons in the 55 to 57 ppm region, which are the N-methyl carbons of quaternary ammonium groups and corroborate the assignment of the 3.24 ppm singlet (the numerical data resolve three lines there, at 56.64, 56.52 and 56.03 ppm on the DSS scale; Section 8.11); the CH_2 trace is dominated by the sugar C-6 carbons and the lysine, arginine, glutamate and succinate methylenes; and the CH trace carries the anomeric and ring carbons of the sugars, the α-carbons and the aromatic CH carbons.

The HSQC (Figure 6) and HMBC (Figure 7) spectra supply the connectivities. In the HMBC the alanine methyl protons at 1.48 ppm correlate to the α-carbon at 51 ppm and to the carboxylate at 176 ppm; the branched-chain methyls at 0.95 to 1.05 ppm correlate to the β- and γ-carbons at 25 to 42 ppm and to the α-carbons at 57 to 60 ppm; the 1.33 ppm doublet correlates both to a carbinol carbon at 69 ppm with a carboxylate at 183 ppm (lactate) and to carbons at 66 and 60 ppm (threonine β and α), so that both lactate and threonine contribute to it; the glutamate γ-methylene at 2.35 ppm correlates to the γ-carboxylate at 181 ppm, the α-carbon at 55 ppm and the β-carbon at 27.7 ppm; the succinate singlet at 2.47 ppm and the acetate singlet at 1.96 ppm each correlate to a carboxylate at 181 to 182 ppm; the 3.24 ppm N-methyl protons correlate to the other N-methyl carbons at 54.5 ppm and to the N-methylene carbon at 68 ppm, the pattern expected for choline or glycerophosphocholine (betaine would give a similar pair but with its methylene carbon near 67 ppm and a carboxylate correlation from a 3.90 ppm singlet, which is not observed); the lysine ε-protons at 3.02 ppm correlate to the δ- and γ-carbons at 27 and 22 ppm; the phenylalanine and tyrosine β-protons at 3.1 to 3.3 ppm correlate to the ring carbons at 127 to 133 ppm and the tyrosine H-2/6 protons at 7.2 ppm to the phenolic C-4 at 155 ppm; the uracil H-6 doublet at 7.85 ppm correlates to C-2 at 152 ppm and C-4 at 165 ppm and the uridine H-1′ at 5.9 ppm to C-6 at 141 ppm, confirming the pyrimidine nucleoside assignment; and the purine H-8 and H-2 singlets at 8.2 to 8.6 ppm correlate to ring carbons between 145 and 156 ppm. The 2D J-resolved spectrum (Figure 8) separates the singlets (formate, the nucleobase H-8/H-2 protons, choline, glycine, succinate, acetate, methionine S-methyl) from the multiplets along the zero-frequency row of the F1 axis and displays the doublet and triplet structure of the amino acid methyls and of the sugar anomeric protons; the tilted responses around the strongly coupled aspartate/asparagine and glutamate/glutamine spin systems are the expected artifacts of J-resolved spectroscopy at 300 MHz.[70] The long-range correlations are assigned individually from the numerical data in Section 8.12, where three of the readings made here are revised: the singlet at 8.60 ppm is the H-ε1 of histidine and not a purine H-8; the trimethylammonium signal is a composite of glycerophosphocholine, choline and betaine, the N-methyl correlations of betaine being present beside those of glycerophosphocholine; and the carbon positions quoted, which were read from Figure 7, lie about 2 to 3 ppm below those measured on the DSS scale.

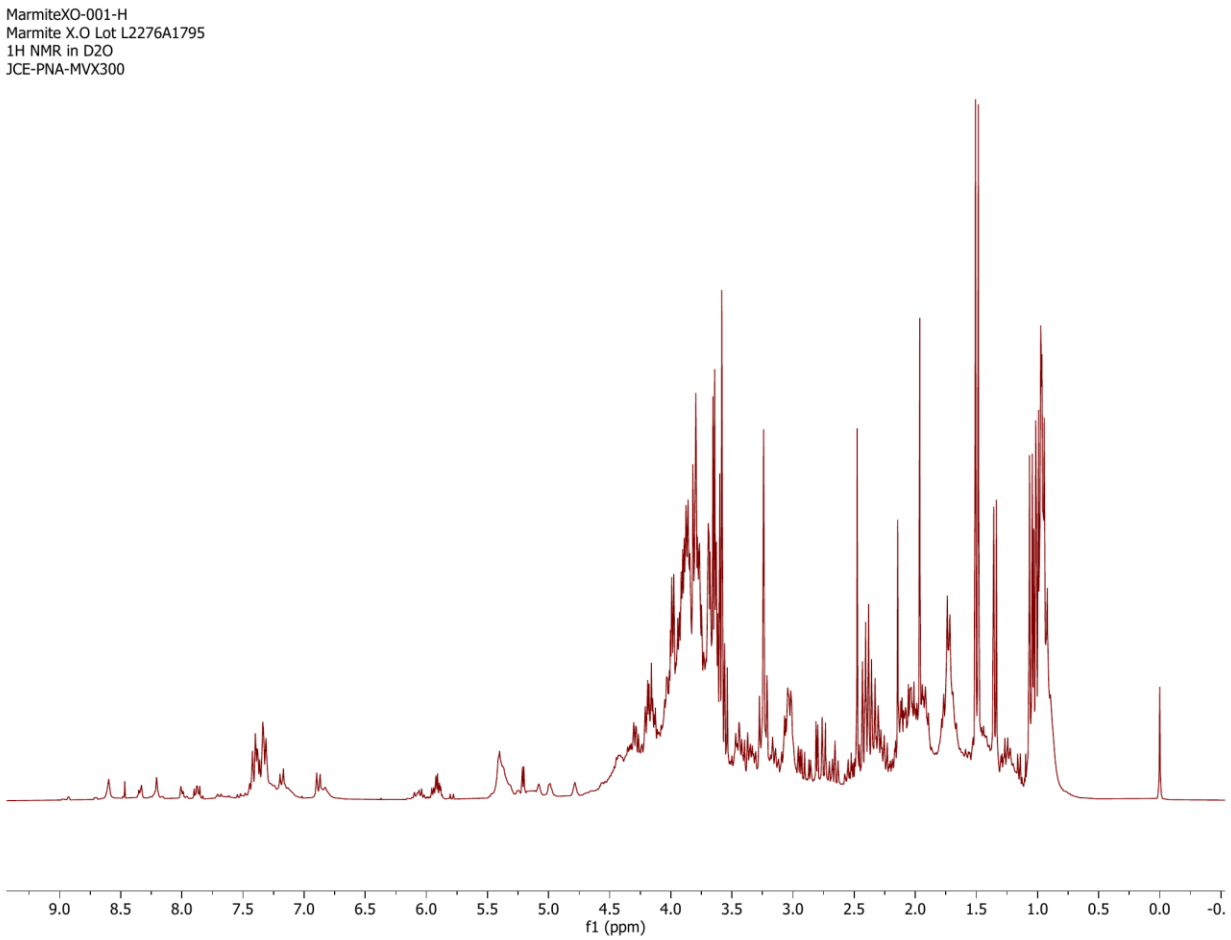


Figure 3. ^{1}H NMR spectrum (300 MHz, D_2O) of Marmite XO, lot L2276A1795.

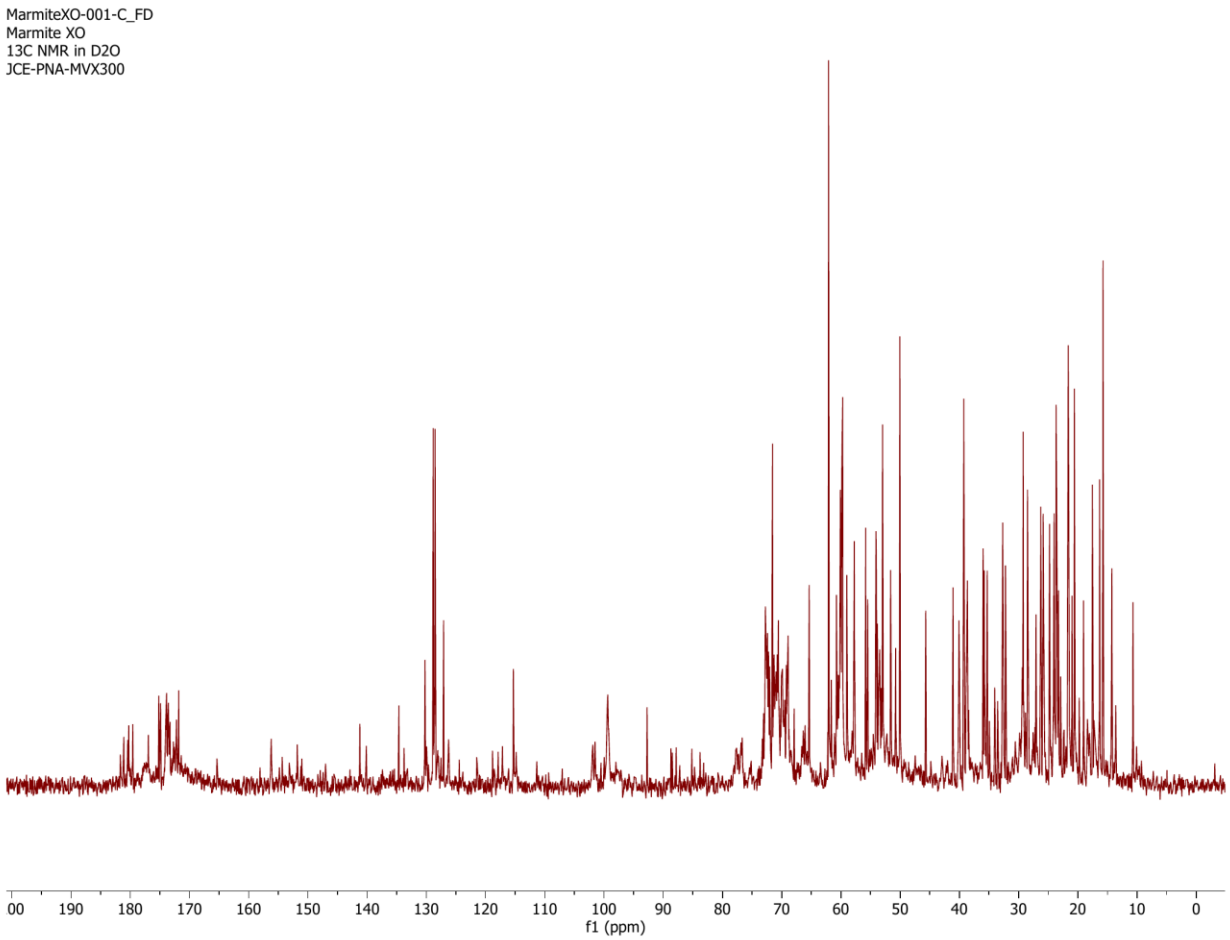


Figure 4. Proton-decoupled ^{13}C NMR spectrum (75 MHz, D_2O) of Marmite XO; the axis reads 3.11 ppm below the DSS scale (alanine CH_3 at 15.75 ppm; Table 8).

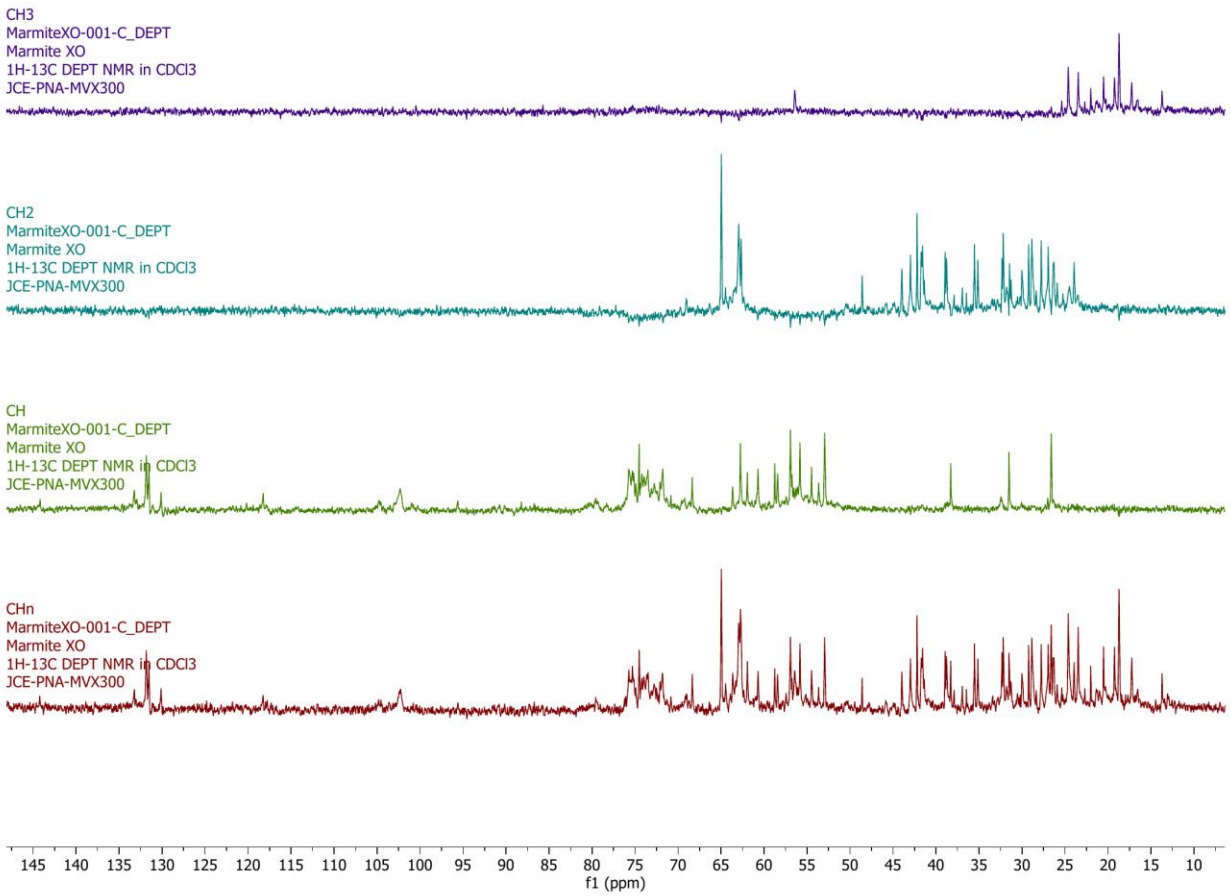


Figure 5. DEPT-edited ^{13}C sub-spectra of Marmite XO: CH_3 (top), CH_2, CH and composite CH_n (bottom). The source caption reads "in $CDCl_3$"; the spectra are those of the D_2O solution, and the axis lies within 0.25 ppm of the DSS scale, about 2.9 ppm above that of Figure 4.

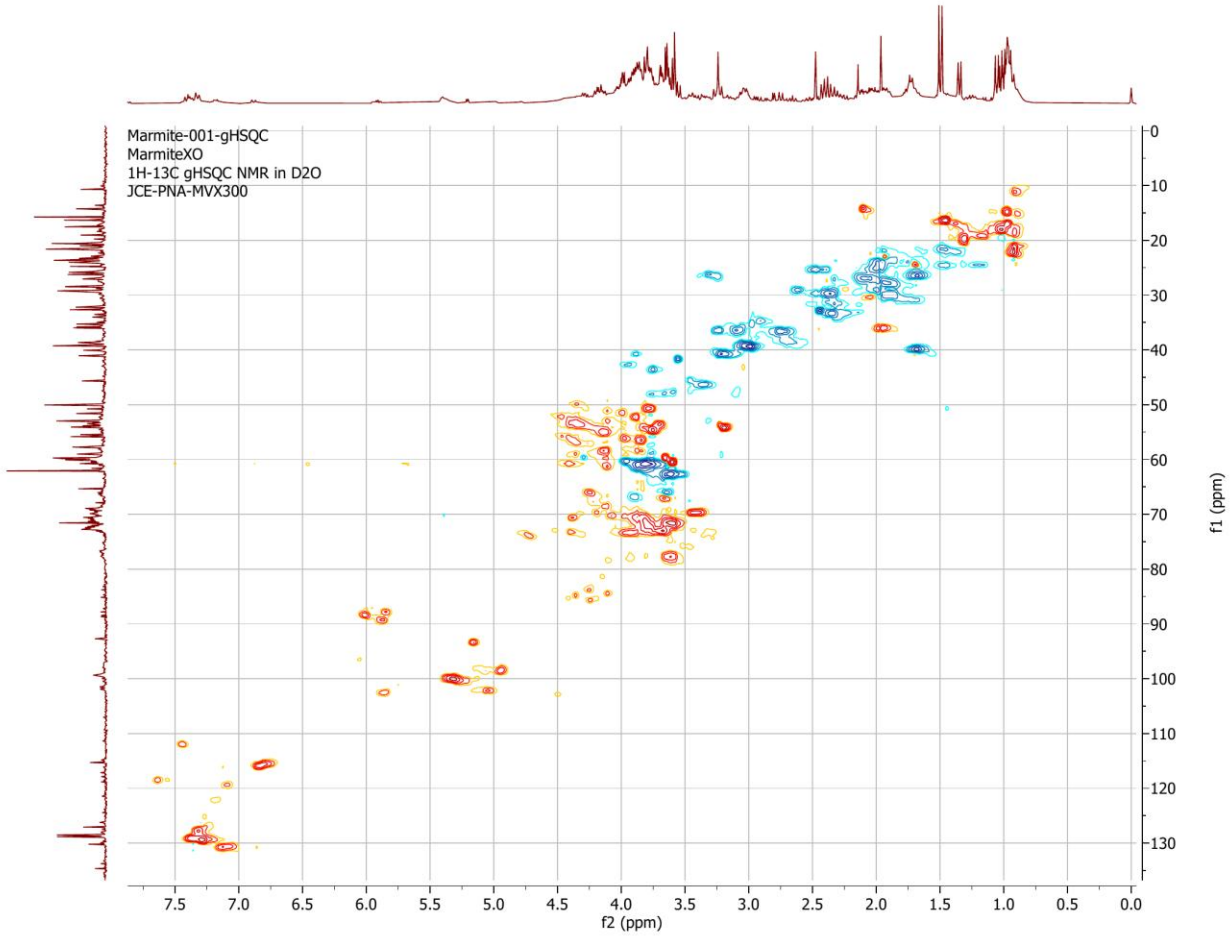


Figure 6. Multiplicity-edited $^{1}H–^{13}C$ gHSQC spectrum (300 MHz, D_2O) of Marmite XO.

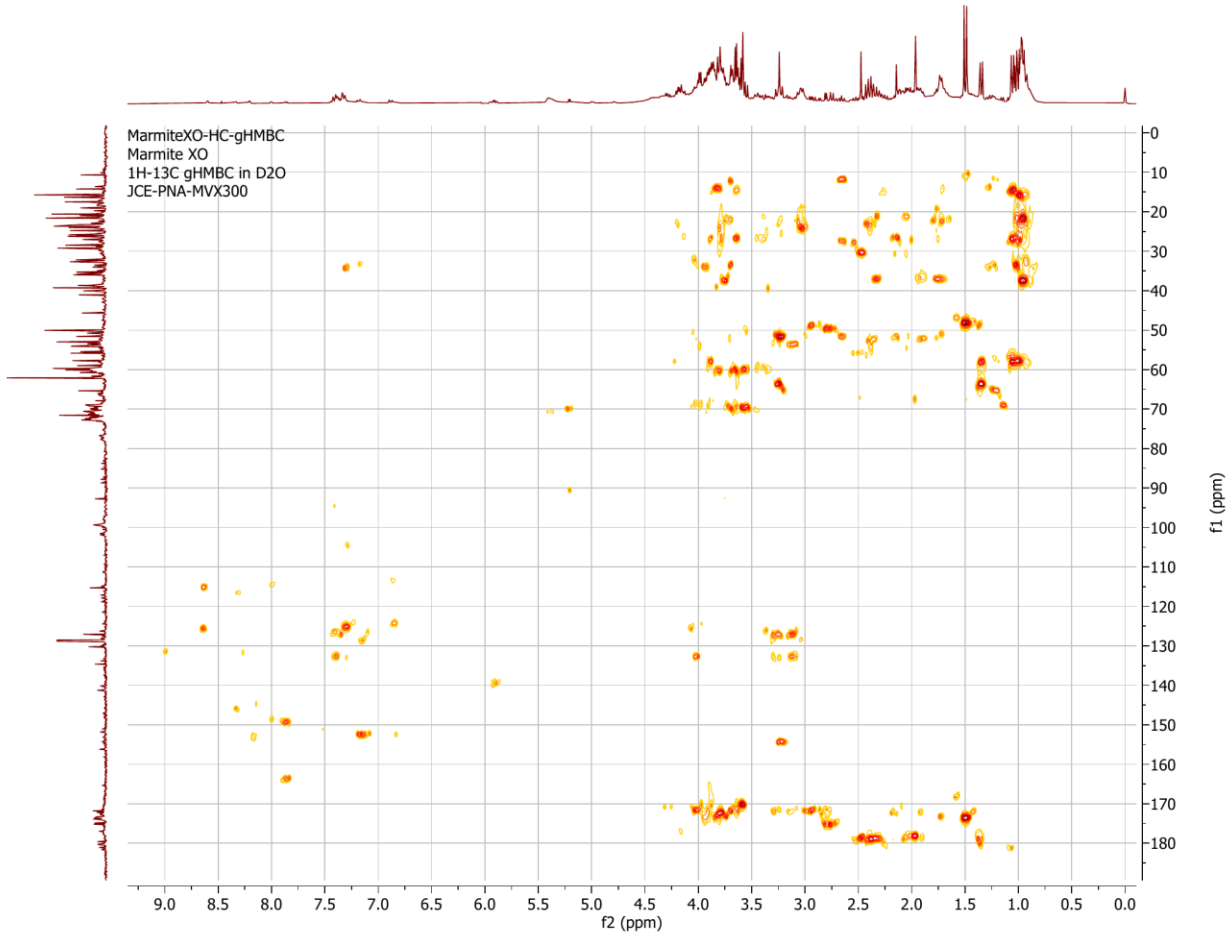


Figure 7. $^{1}H–^{13}C$ gHMBC spectrum (300 MHz, D_2O) of Marmite XO.

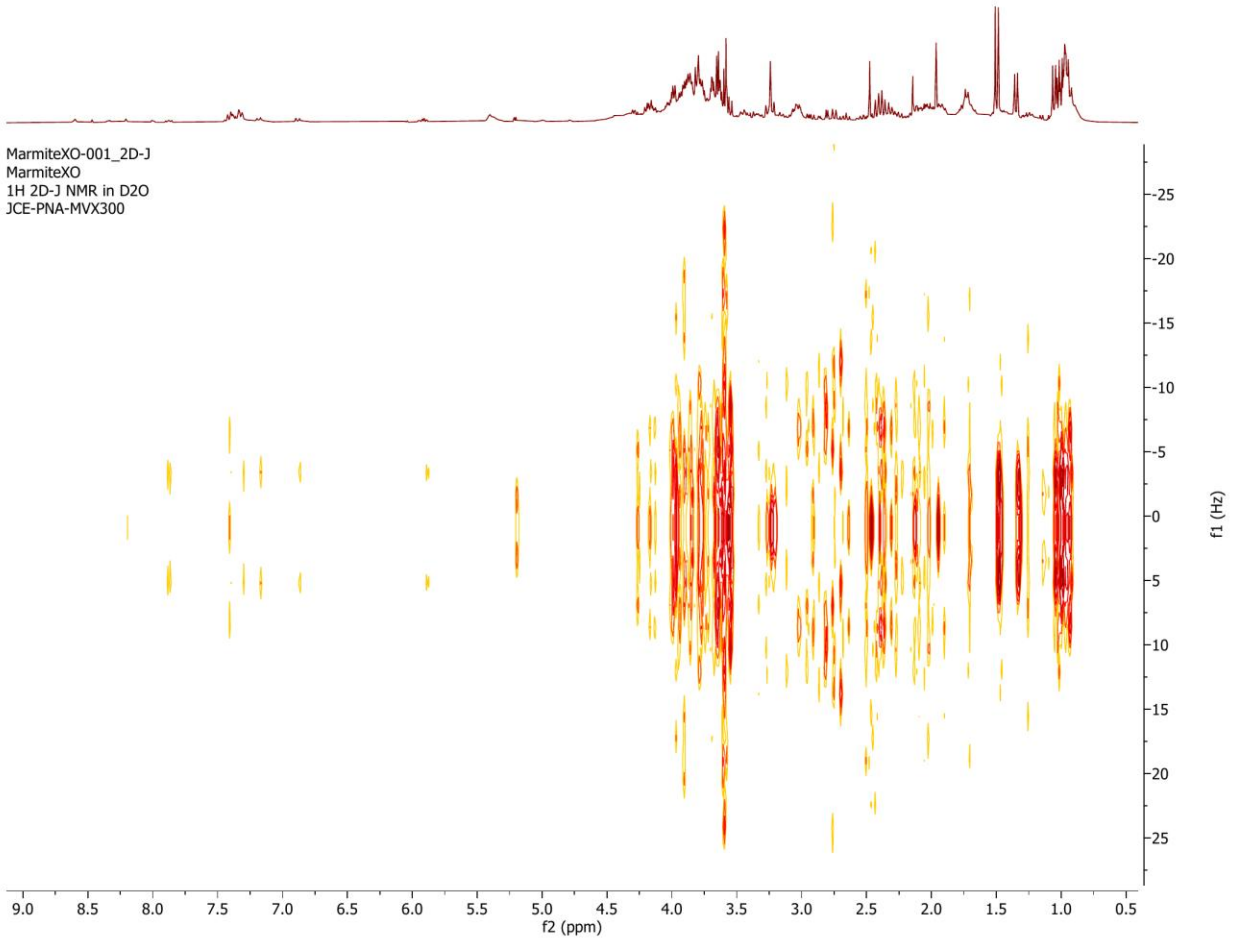


Figure 8. Homonuclear 2D J-resolved ^{1}H NMR spectrum (300 MHz, D_2O) of Marmite XO; F1 in Hz.

8.5 Vegemite

Vegemite (Figure 9) shares the amino acid, organic acid and choline inventory of the British product — the alanine doublet is again the tallest signal, and glycine (3.56 ppm), lysine, glutamate, succinate (here at 2.41 ppm, indicating a solution pH closer to neutral than for Marmite), acetate, the lactate/threonine doublet and the branched-chain methyls are all prominent — but it differs in three respects that together constitute a product fingerprint. First, the anomeric region is dominated by a sharp doublet at 5.19 ppm that is the strongest signal between 4.5 and 9.5 ppm; the HSQC (Figure 12) correlates it to a carbon at 93.5 ppm (TMS scale), the ^{13}C spectrum (Figure 10; DSS scale) shows the anomeric carbon at 96.0 ppm as the tallest sugar CH signal with the four ring carbons at 75.3, 74.8, 73.8 and 72.5 ppm and the CH_2 carbon at 63.3 ppm, the DEPT sub-spectra (Figure 11) confirm the CH multiplicity of the anomeric and ring carbons and the CH_2 multiplicity of the 63 ppm carbon, and the HMBC (Figure 13) shows the H-1 proton correlating both to an anomeric carbon at 93 ppm — which can only be the C-1 of the partner ring, reached across the glycosidic oxygen — and to the ring carbons at 71 to 72 ppm. These values match α,α-trehalose (96.0, 75.2, 74.8, 73.9, 72.4 and 63.4 ppm on the DSS scale) to within 0.2 ppm, and the symmetric inter-residue HMBC correlation excludes maltose, isomaltose, sucrose and the reducing sugars. Vegemite is therefore, on this evidence, a trehalose-rich extract, which is a direct record of the physiological state of the brewer's yeast at harvest and of an autolysis regime under which trehalase action was limited; the origin and the sensory consequences of that trehalose are taken up in Section 8.9.[37,38,53] Second, the α-(1→4)-glucan envelope at 5.40 ppm and the reducing-sugar anomeric signals are weak: a small doublet near 5.4 ppm with an HSQC correlation at 100 ppm is the only trace of the declared malt extract, and weak ^{13}C signals at 103 to 105.5

ppm (DSS scale) are the only evidence of higher glucan or mannan. Third, the nucleobase region between 8.0 and 8.65 ppm, while present, is markedly less intense than in the British products, the nucleoside H-1′ cross-peak at δH 5.97/δC 89 in the HSQC is weak, and the aromatic amino acid signals are also somewhat weaker relative to the aliphatic ones. The formate singlet at 8.46 ppm and a weak niacin H-2 singlet at 8.94 ppm are present.

The ^{13}C spectrum of Vegemite is otherwise closely similar to that of Marmite XO once the 3 ppm difference between the two axes is allowed for: carboxylates at 183.5 to 184.5 ppm and 176 to 177.5 ppm, amide carbonyls at 172 to 174 ppm, the arginine guanidino carbon at 159.5 ppm, aromatic carbons at 130 to 133.3 ppm, tyrosine C-3/5 at 118.5 ppm, glutamate C-α at 57.4 ppm, the choline N-methyl at 56.6 ppm, alanine C-α at 53.4 ppm, glycine at 44.4 ppm, lysine C-ε at 42.1 ppm, glutamate C-γ at 36.3 ppm and C-β at 29.8 ppm, the leucine, valine, isoleucine, threonine and lactate methyls between 13.9 and 24.9 ppm and the alanine methyl at 19.0 ppm. In the HMBC the doublet at 1.33 ppm correlates to a methine carbon at 69 ppm and to a carboxylate at 183 ppm, which is the pattern of lactate rather than of threonine, so that lactate is confirmed as a real constituent of this product; threonine may co-resonate. The 3.24 ppm singlet correlates to its N-methyl and N-methylene carbons as in Marmite XO (56.6 and 68.4 ppm on the DSS scale), which are those of glycerophosphocholine (Section 8.12). A weak positive cross-peak at δH 5.35/δC 130 in the HSQC, which has no counterpart in the two British spectra, lies where the olefinic CH=CH carbons of unsaturated fatty acyl chains resonate and is tentatively attributed to a trace of free or esterified unsaturated fatty acid from the yeast lipid; it would be confirmed by the accompanying allylic CH_2 cross-peak at δH 2.0/δC 27 and by the terminal methyl at 0.88/14. The allylic methylene (2.02/29.8) and the chain methylene (1.32/31.9) correlations were both found when the numerical data were examined, with the olefinic correlation at 5.31/132.0, and the assignment is no longer tentative (Section 8.11).

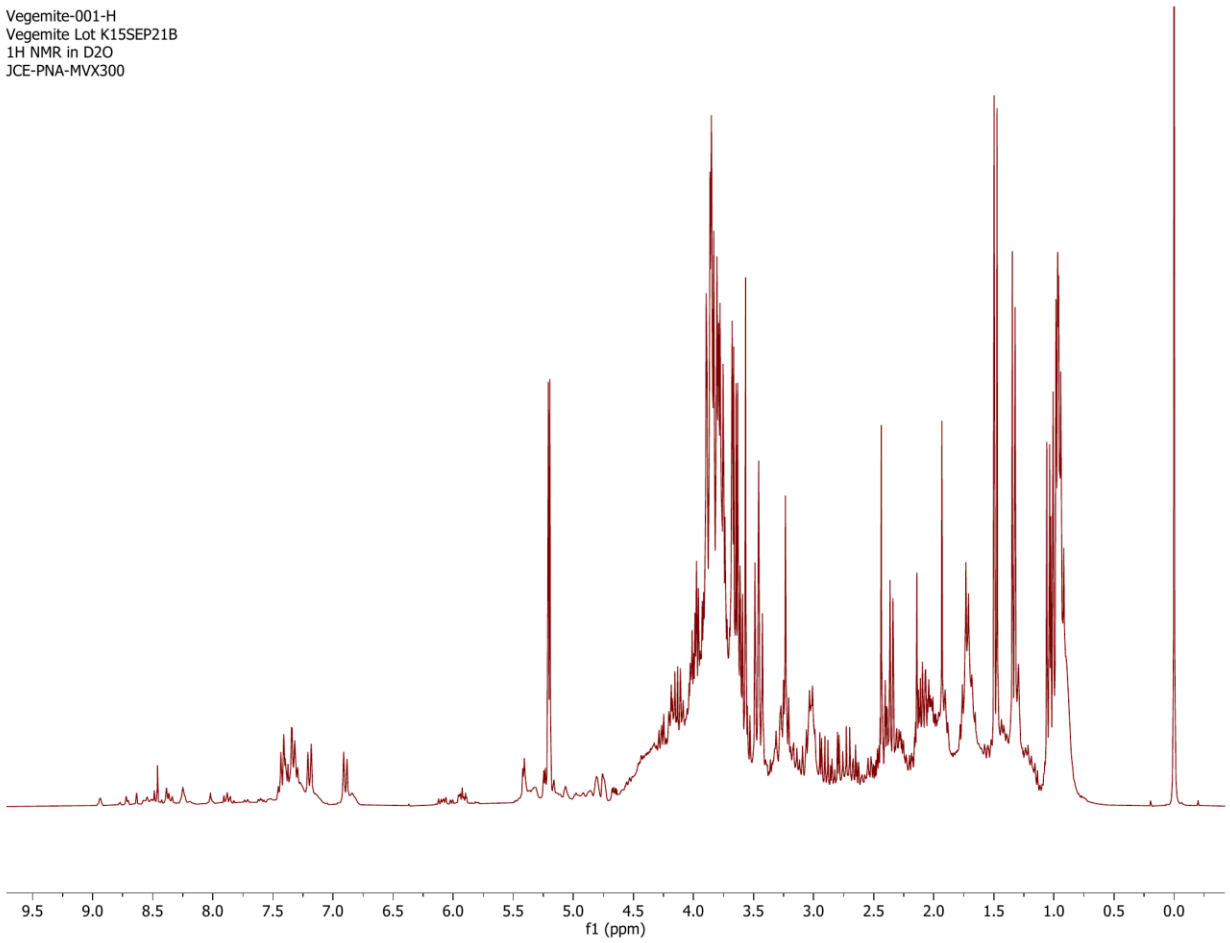


Figure 9. ^{1}H NMR spectrum (300 MHz, D_2O) of Vegemite, lot K15SEP21B.

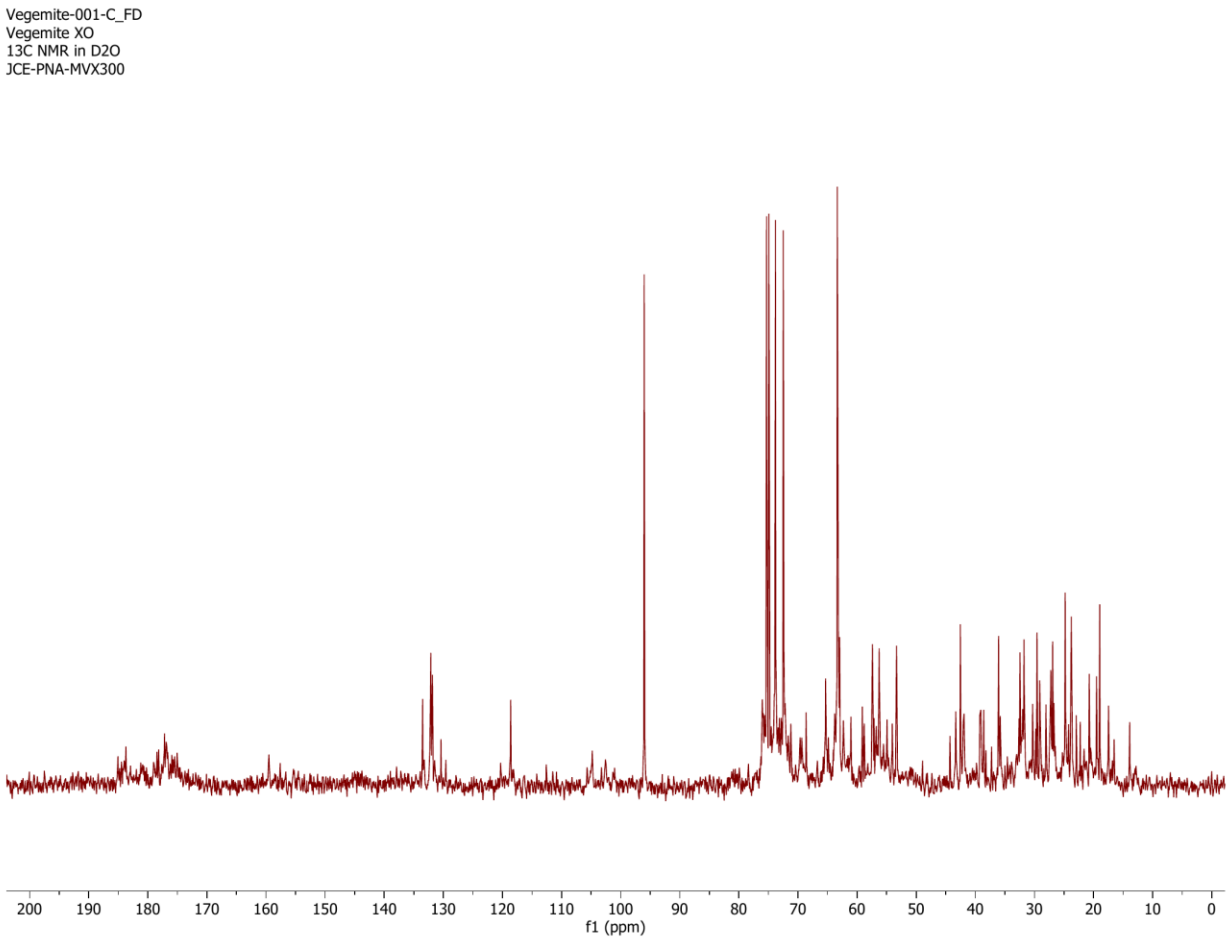


Figure 10. Proton-decoupled ^{13}C NMR spectrum (75 MHz, D_2O) of Vegemite; DSS scale (alanine CH_3 at 19.0 ppm). The source figure is captioned "Vegemite XO"; the sample is Vegemite.

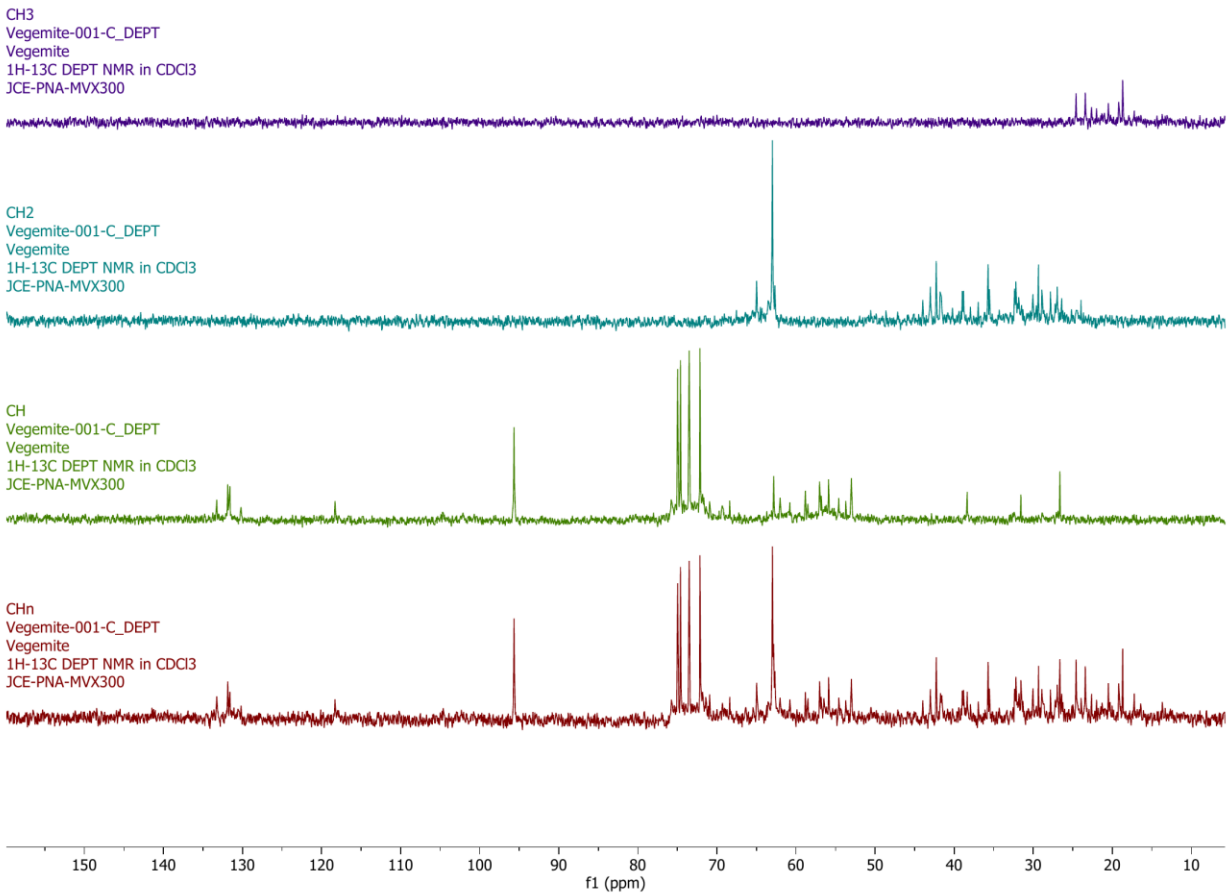


Figure 11. DEPT-edited ^{13}C sub-spectra of Vegemite: CH_3 (top), CH_2, CH and composite CH_n (bottom).

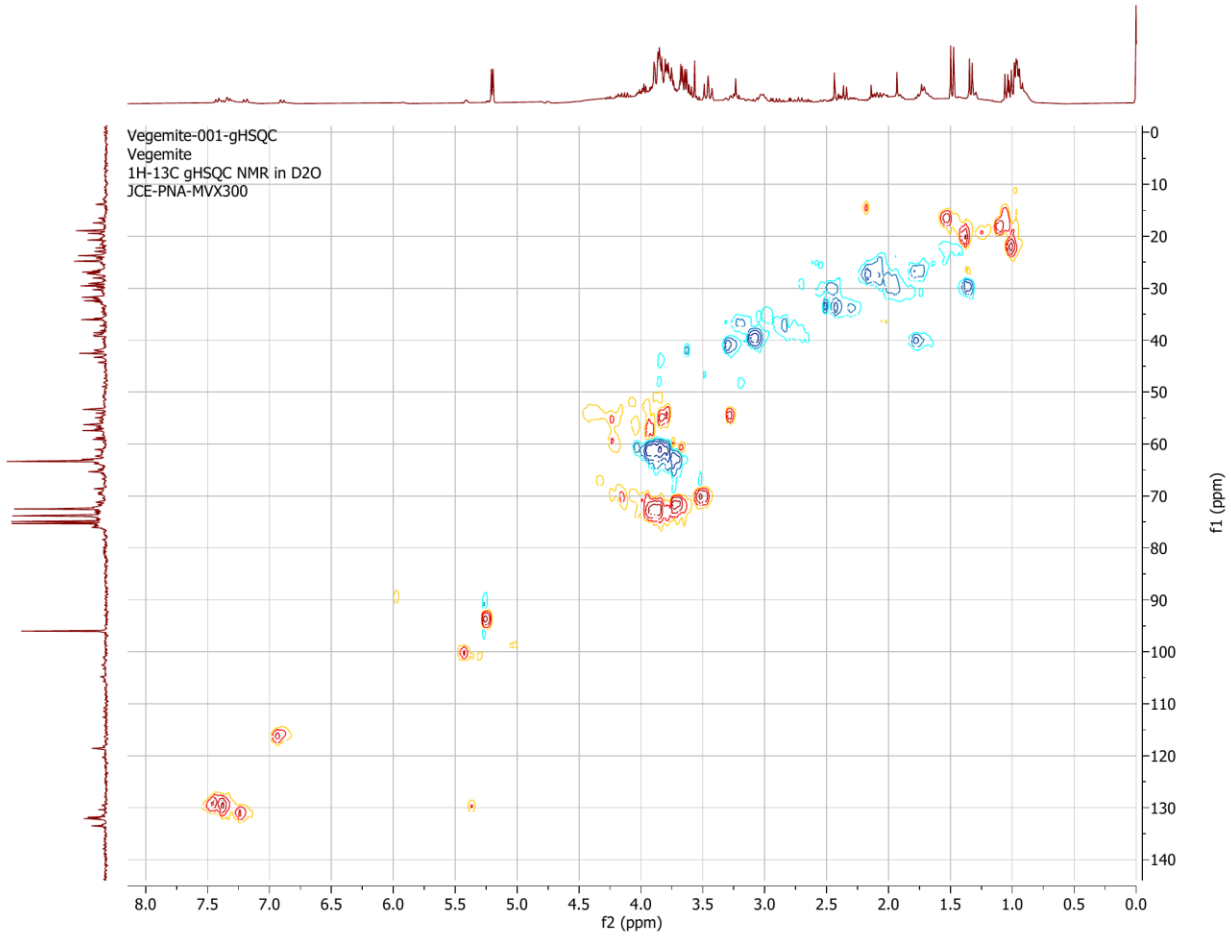


Figure 12. Multiplicity-edited ^{1}H–^{13}C gHSQC spectrum (300 MHz, D_2O) of Vegemite.

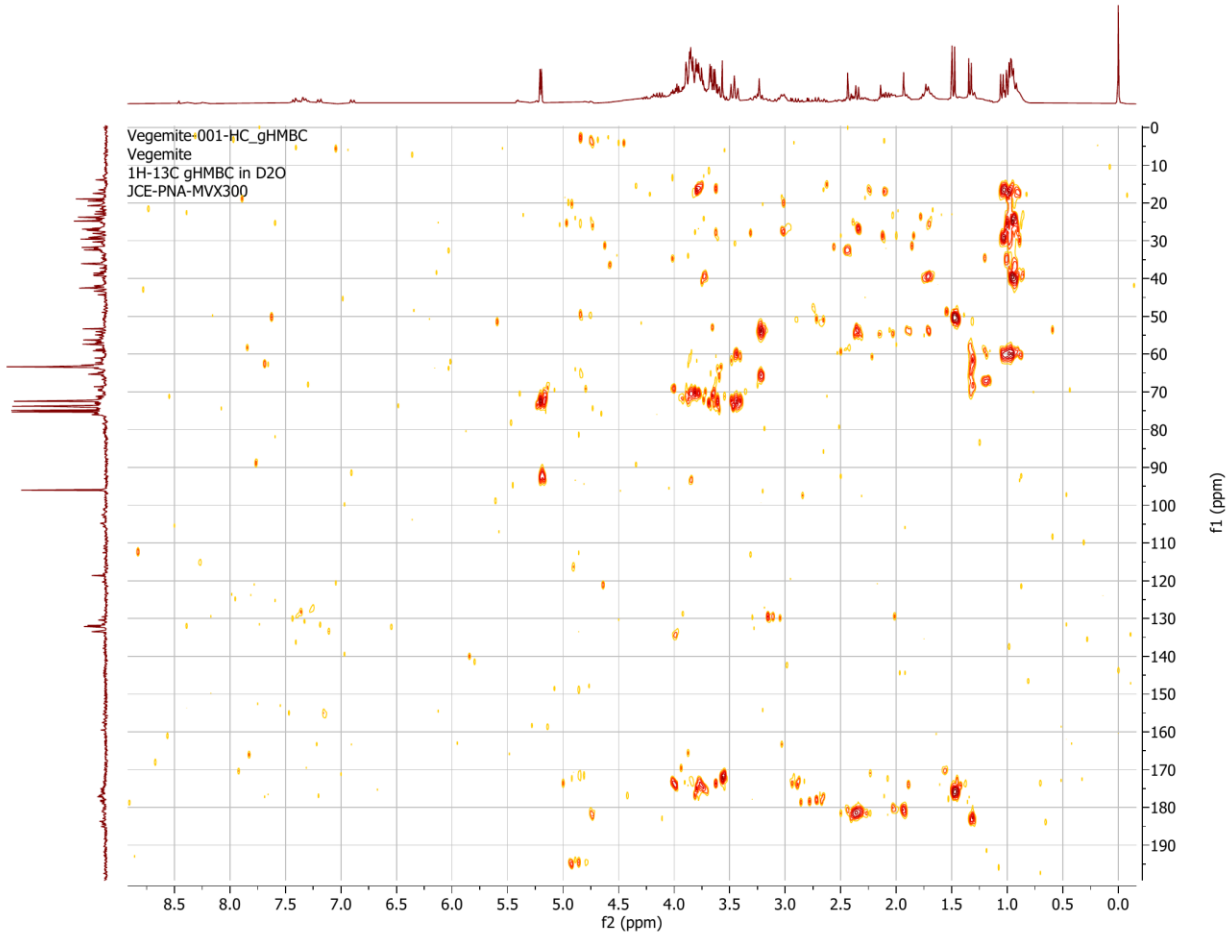


Figure 13. ^{1}H–^{13}C gHMBC spectrum (300 MHz, D_2O) of Vegemite. The vertical streak of weak responses at δH 4.8 is t_1 noise at the residual water frequency.

8.6 Sanitarium Marmite (New Zealand)

The New Zealand product (Figure 14) is a different spectrum altogether, and the reason is on the label. The spectrum is dominated by sucrose: the glucosyl H-1 doublet at 5.41 ppm, the fructosyl H-3′ doublet at 4.22 ppm, the H-4′ triplet at 4.05 ppm and the H-1′ singlet at 3.67 ppm are the most intense signals in the spectrum, and the ^{13}C spectrum (Figure 15) shows the full sucrose carbon set — the quaternary C-2′ at 103.3 ppm (absent, as it must be, from the DEPT sub-spectra of Figure 16), C-1 at 92.0 ppm, C-5′ at 81.2 ppm, C-3′ and C-4′ at 76.3 and 74.0 ppm, the glucosyl ring carbons at 69.2 to 72.6 ppm, and the three methylene carbons C-6′, C-1′ and C-6 at 62.3, 61.4 and 60.1 ppm, all 2.9 ppm below their DSS-scale values (about 1 ppm below the TMS-scale literature values) because of the referencing of this spectrum. The three CH_2 carbons stand out as the dominant negative-phase cross-peaks in the HSQC (Figure 17) at δH 3.6 to 3.85/δC 61 to 63, the H-3′ and H-4′ protons correlate to carbons at 76.5 and 74.5 ppm, and the H-1 proton at 5.41 ppm correlates to C-1 at 92.5 ppm. The HMBC (Figure 18) shows the H-1 proton correlating across the glycosidic oxygen to the C-2′ carbon and to the glucosyl ring carbons at 70 to 72 ppm, and the H-1′ singlet at 3.67 ppm correlating to C-2′ and to C-3′, the diagnostic inter-residue correlations of sucrose.[53] Beneath the sucrose, an α-glucan — taken in version 1.1 to be the declared corn maltodextrin — is visible as a broad anomeric shoulder near 5.4 ppm with an HSQC correlation at 100 ppm, a branch-point cross-peak at δH 4.95/δC 98.5 and ^{13}C signals at 99.6 and 98.0 ppm; a weak signal at 96.0 ppm with a doublet near 4.65 ppm marks the β-anomer of a reducing glucose end, and a sharp doublet at 5.22 ppm is consistent with either a small amount of trehalose or the α-reducing end of the maltodextrin. The numerical data

resolve these points (Section 8.11): trehalose is present, at about one molecule for every ten of sucrose, beside the α-anomer of free glucose, which arises with free fructose from the inversion of about one-eighth of the sucrose; and the α-glucan has the branch proportion and the glucan-to-trehalose ratio of the yeast products to which no maltodextrin is added, so that an added maltodextrin is not distinguishable within it.

The yeast-derived constituents are all present but are compressed by the dynamic range of the sucrose signals: the alanine doublet (HSQC 1.48/17.0), the branched-chain methyls, the lactate/threonine doublet, succinate (at 2.47 ppm, again indicating a mildly acidic solution), acetate, the choline singlet at 3.24 ppm (HSQC 3.24/54.5), lysine, glutamate, glycine, the tyrosine and phenylalanine aromatics (HSQC 6.9/116, 7.2/130.5, 7.4/129) and a weak nucleobase and formate region are all identifiable, and the CH_3 and CH_2 DEPT sub-spectra reproduce the amino acid methyl and methylene carbons at reduced intensity. The HMBC confirms alanine (1.48 → 51 and 174 ppm), glutamate (2.35 → 180 ppm), succinate (2.45 → 180 ppm) and acetate (1.92 → 180 ppm) among the minor constituents; the weak responses stacked at δH 4.8 are t_1 noise at the water frequency and carry no chemical information. A weak triplet near 1.18 ppm is consistent with a trace of ethanol. The declared iron does not produce obvious line broadening at this loading, but the linewidths of the reference and of the sharp singlets should be compared against a non-fortified product before quantitative work is undertaken. The title under which these spectra were originally circulated within PNA — "NMR beauty spoiled by maltodextrin" — captures the analytical point but misnames the principal culprit: the carbohydrate that dominates this spectrum is the declared sugar, sucrose, and the numerical analysis of version 1.2 finds no α-glucan beyond what the yeast extract itself would bring (Section 8.11). Quantitation of the yeast-derived fraction in such a product requires either a higher receiver dynamic range, selective suppression of the sucrose resonances, or the use of ^{13}C or HSQC intensities in which the sucrose signals are better dispersed.

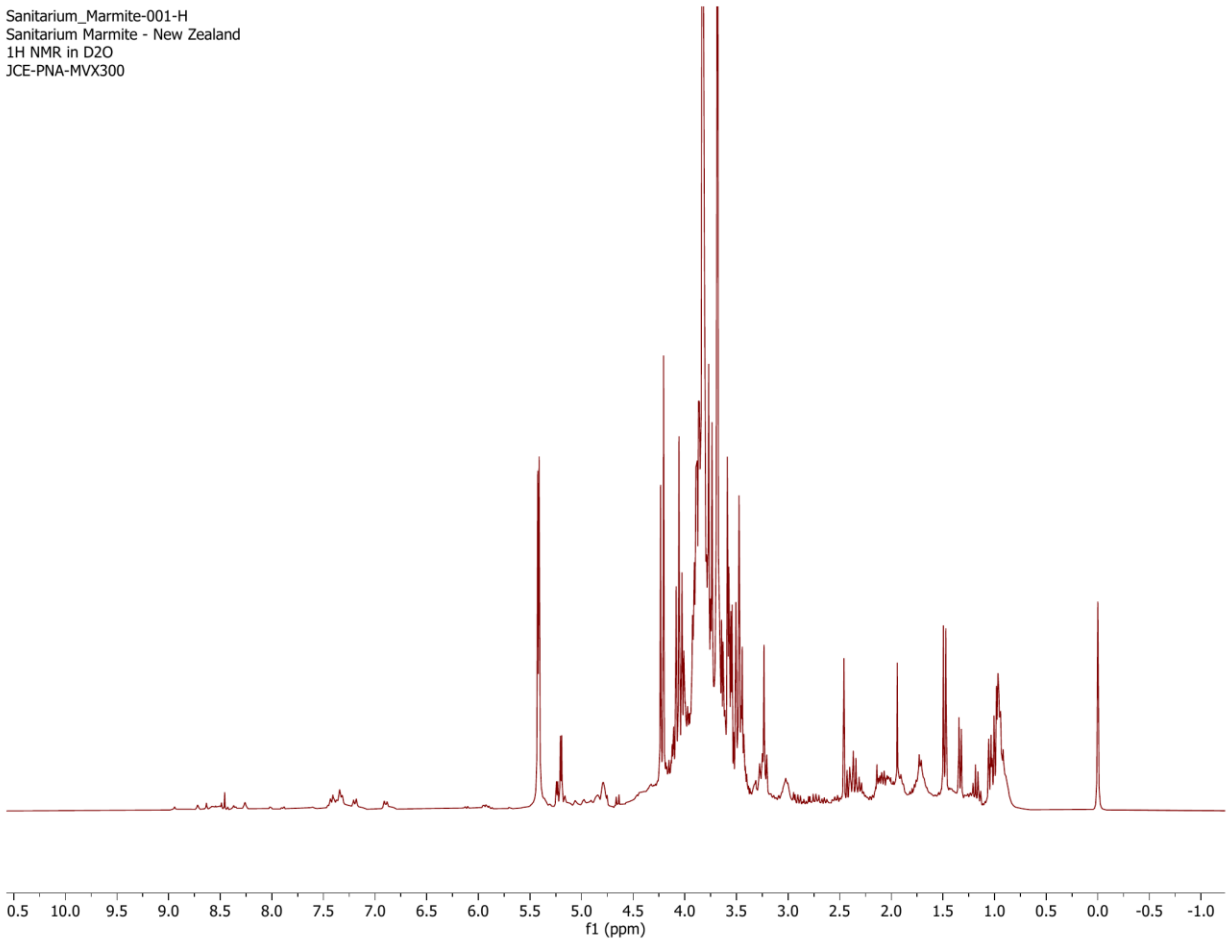


Figure 14. ^{1}H NMR spectrum (300 MHz, D_2O) of Sanitarium Marmite (New Zealand).

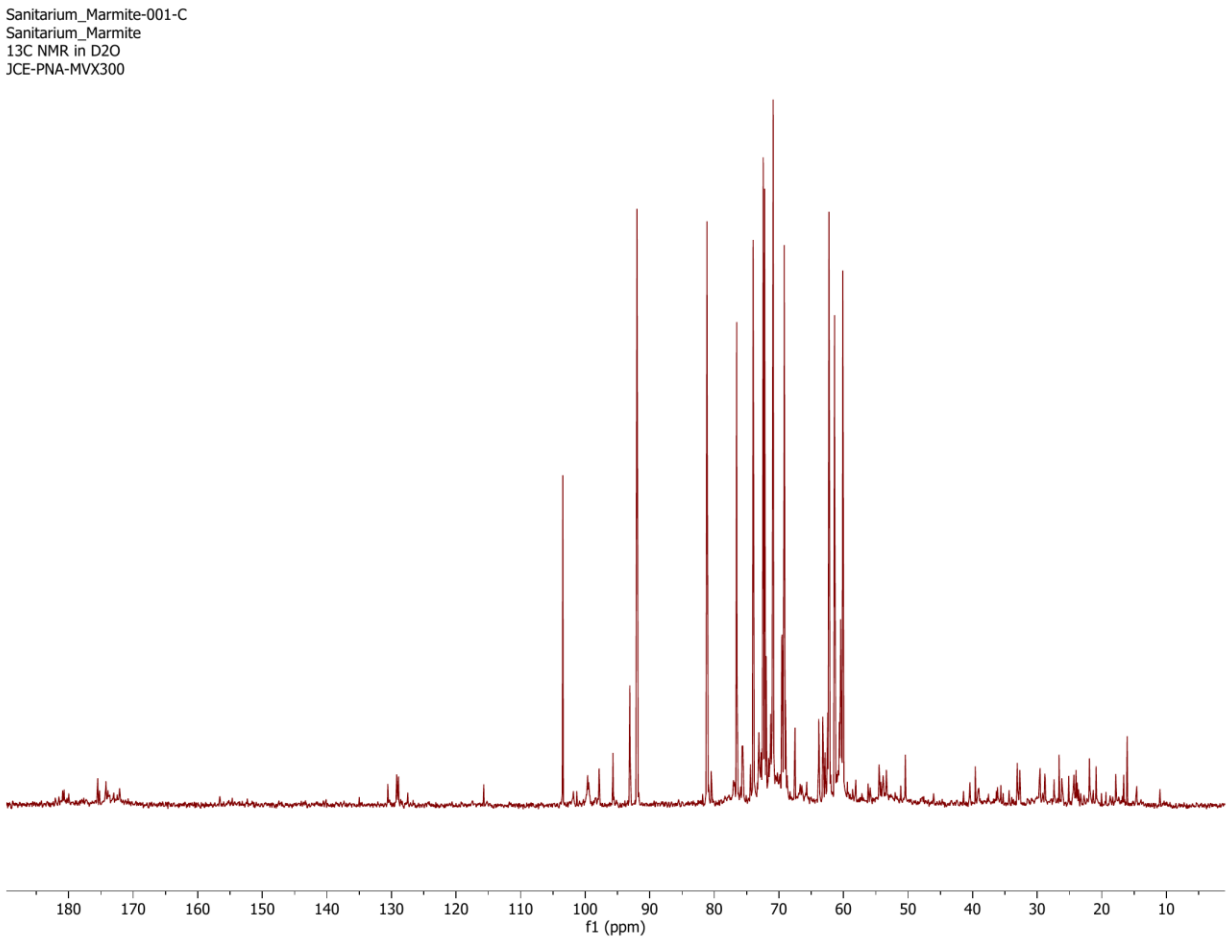


Figure 15. Proton-decoupled ^{13}C NMR spectrum (75 MHz, D_2O) of Sanitarium Marmite; the axis reads 2.91 ppm below the DSS scale (sucrose C-2′ at 103.45 ppm; Table 8).

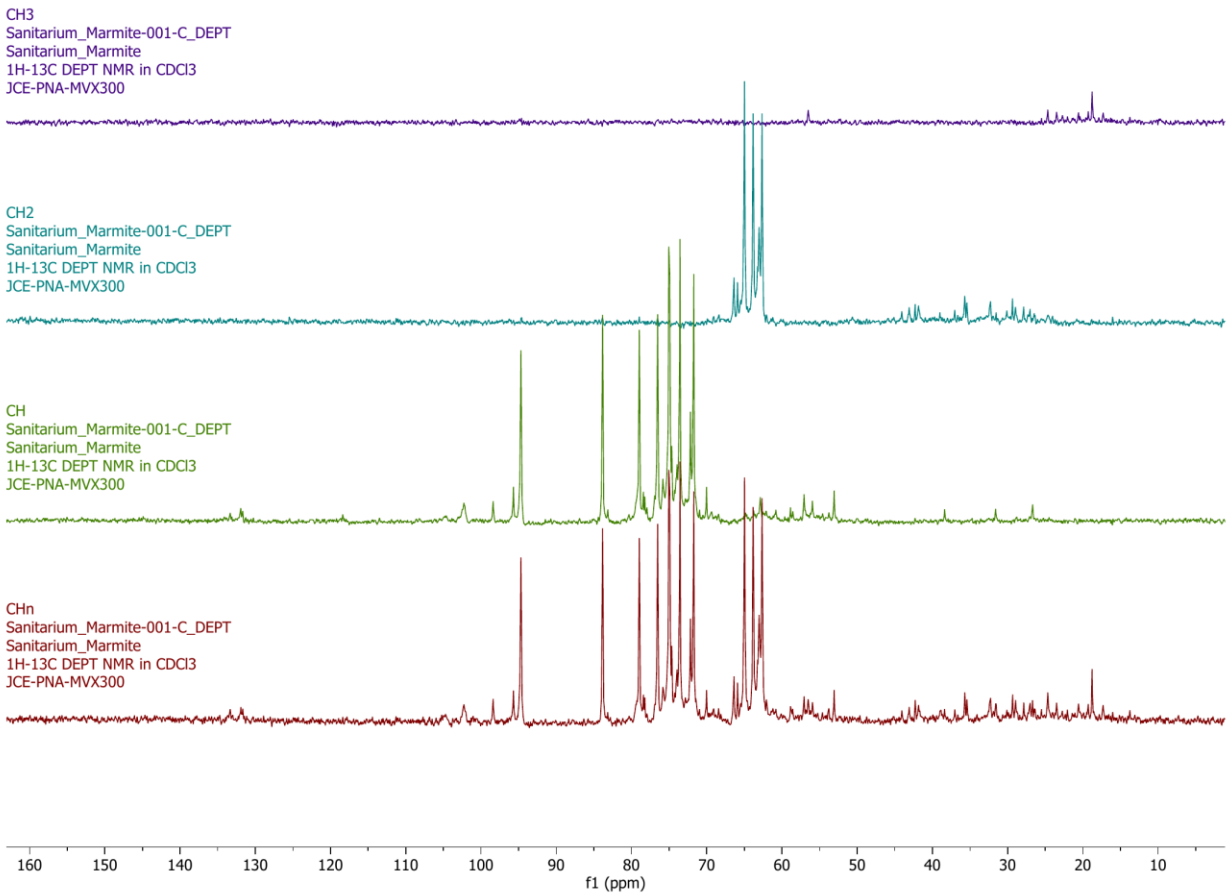


Figure 16. DEPT-edited ^{13}C sub-spectra of Sanitarium Marmite: CH_3 (top), CH_2, CH and composite CH_n (bottom).

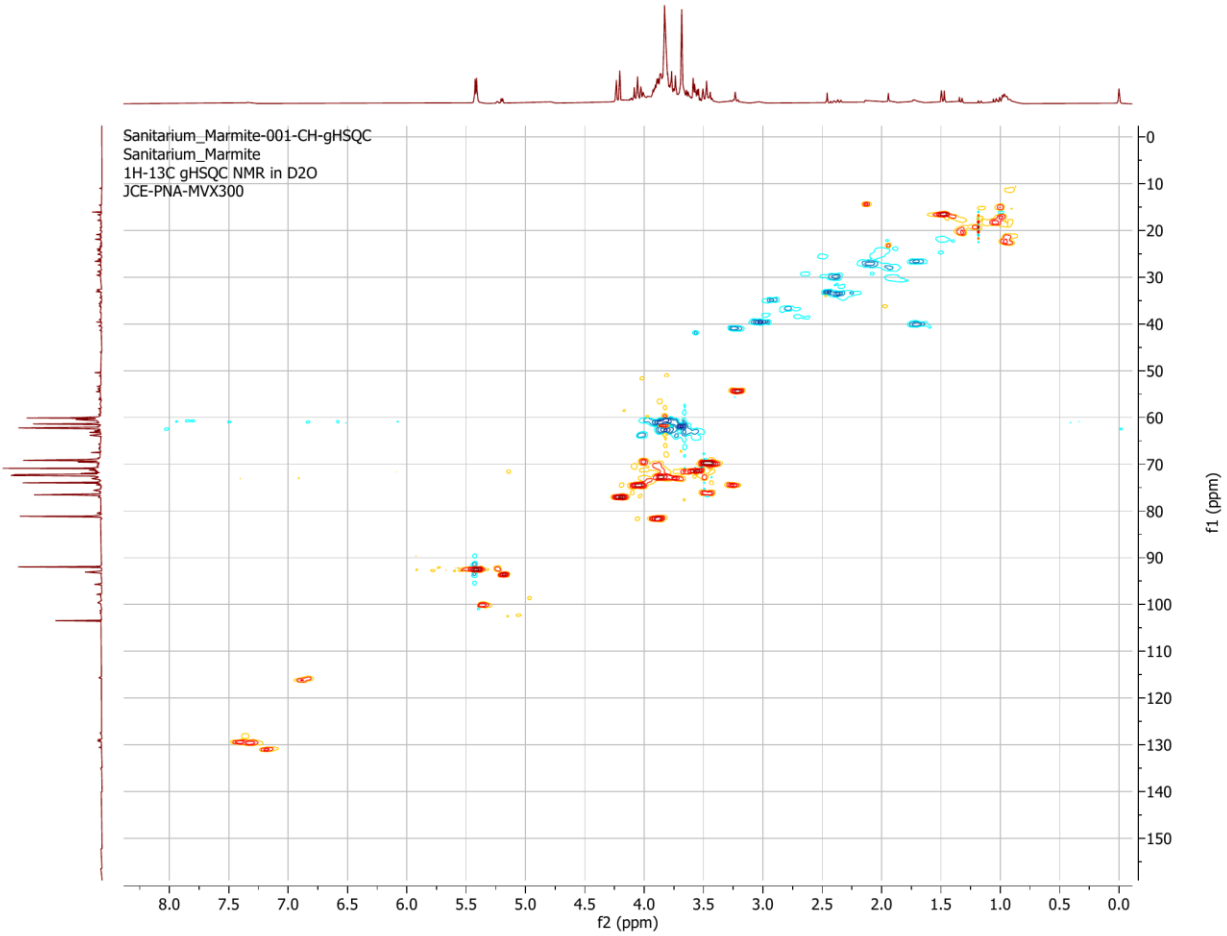


Figure 17. Multiplicity-edited $^{1}H–^{13}C$ gHSQC spectrum (300 MHz, D_2O) of Sanitarium Marmite.

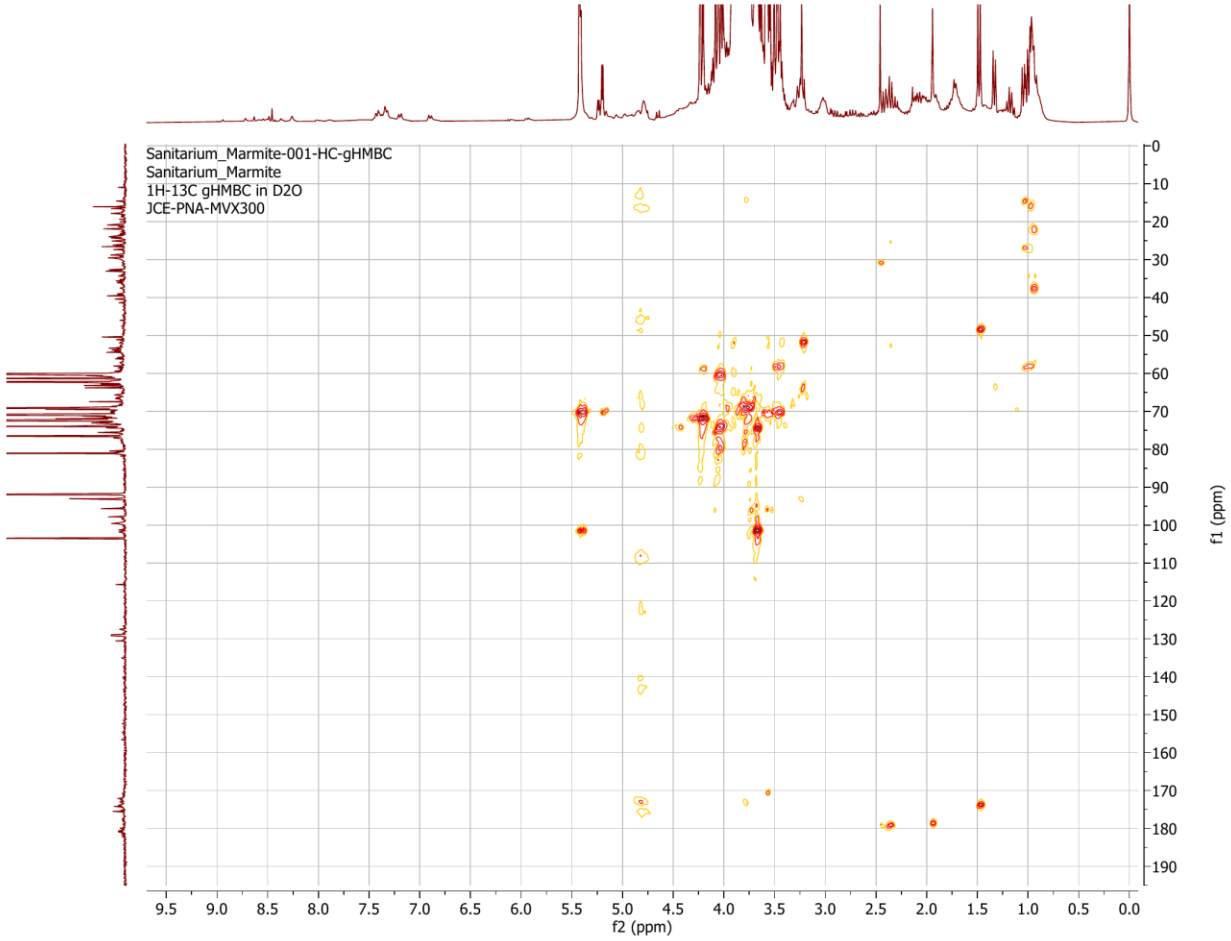


Figure 18. ^{1}H–^{13}C gHMBC spectrum (300 MHz, D_2O) of Sanitarium Marmite. The column of weak responses at δH 4.8 is t_1 noise at the residual water frequency.

8.7 Promite

Promite (Figures 19 to 21) is not a yeast-extract spectrum, and with the ^{13}C and HSQC data now available the conclusion no longer rests on the ^{1}H spectrum alone. Sucrose dominates the ^{1}H spectrum (5.41, 4.22, 4.05 and 3.67 ppm) and the ^{13}C spectrum (Figure 20: 103.7, 92.0, 81.3, 76.4, 74.0, 72.5, 72.3, 71.0, 69.3, 62.3, 61.4 and 60.2 ppm, on the same slightly offset scale as the New Zealand spectrum), and the α- and β-glucose anomeric signals (^{1}H 5.23 and 4.65 ppm; ^{13}C 92 and 96.0 ppm; HSQC 5.23/92 and 4.65/96) are consistent with the declared glucose syrup, whose maltooligosaccharides also account for the α-(1→4) anomeric signals at 99.6 ppm and δH 5.4/δC 99.5. The amino acid region is present but weak — alanine (HSQC 1.48/17.0; ^{13}C 51.5 and 16.5 ppm), glutamate (^{13}C 54.8, 33 and 27.5 ppm), aspartate/asparagine β-methylenes (HSQC 2.75 to 2.8/37 to 39) and small phenylalanine and tyrosine cross-peaks (7.4/129, 7.2/130.5, 6.9/116) are identifiable, in proportion to a declared protein content of 13 g per 100 g against 26 to 34 g per 100 g for the yeast products. Decisively, the purine and pyrimidine region of the ^{1}H spectrum between 7.8 and 8.7 ppm and the nucleoside H-1′ region between 5.9 and 6.2 ppm are essentially empty; the HSQC has no cross-peak in the δH 5.9 to 6.2/δC 87 to 90 window where every yeast product in this set shows the nucleoside C-1′ correlation; and the ^{13}C spectrum contains no signal between 135 and 170 ppm, the region in which the purine and pyrimidine ring carbons of the yeast products (141 to 157 ppm) and their arginine guanidino carbon are found. A vegetable protein hydrolysate contains no RNA and therefore no nucleotides; a yeast extract cannot be made without them.

Three further signals point to the acid-hydrolysis origin of the protein component. The sharp singlet at 8.45 ppm, which in this spectrum is the strongest signal downfield of 5.5 ppm, is formate; a sharp singlet at 2.22 ppm with an HSQC correlation to a methyl carbon at 29.5 ppm, accompanied by triplets near 2.42 and 2.75 ppm, is consistent with levulinate (methyl carbon 29.6 ppm), although acetone (2.22 ppm, 30.9 ppm) cannot be excluded from these data alone; and formic and levulinic acids are the equimolar end-products of the acid-catalyzed degradation of hexoses through 5-hydroxymethylfurfural that accompanies the hydrochloric acid hydrolysis of carbohydrate-containing protein sources. Levulinic acid is the established chromatographic marker of acid-hydrolyzed vegetable protein in soy sauce, where a regulatory threshold of 1 mg/mL has been used to identify its addition, and the same chemistry would be expected in any spread based on acid-HVP.[64,65] The levulinate assignment would be settled by an HMBC correlation from the 2.22 ppm singlet to the ketone carbon near 216 ppm, for which no gHMBC spectrum of Promite is available, or by spiking. The statement of version 1.1 that the ketone carbon lies outside the window of Figure 20 was incorrect: the ^{13}C spectrum extends to 271 ppm, and no line is detected above the noise in the ketone region, which for an unprotonated carbonyl carbon of a minor constituent is not evidence of absence. The gHSQC nonetheless supplies all three protonated carbons of levulinate, and acetone is excluded as the source of the pattern (Section 8.11). An AB quartet at 2.53 and 2.66 ppm with a negative HSQC cross-peak at δH 2.6/δC 46 and a ^{13}C signal at 46.5 ppm is the methylene of the declared citric acid (46.3 ppm), and the carboxyl signals at 181, 174.5 and 171 ppm are consistent with citrate, glutamate and peptide carbonyls. A strong choline-type singlet is absent, in keeping with the minor role of yeast extract in the formulation, and small features near 3.55 to 3.8 ppm may include glycerol from the glycerol monostearate emulsifier.

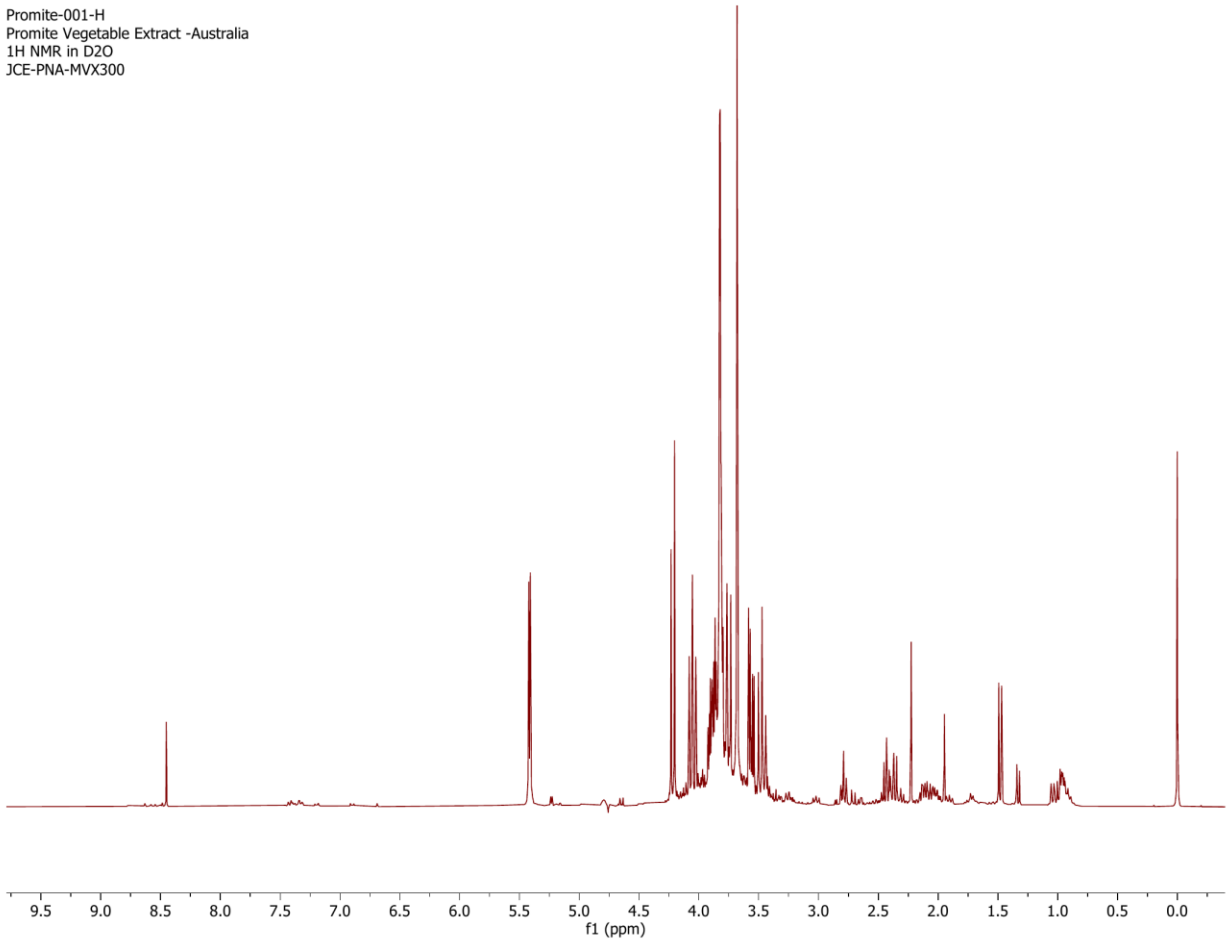


Figure 19. ^1H NMR spectrum (300 MHz, D_2O) of Promite (Mars Food, Australia).

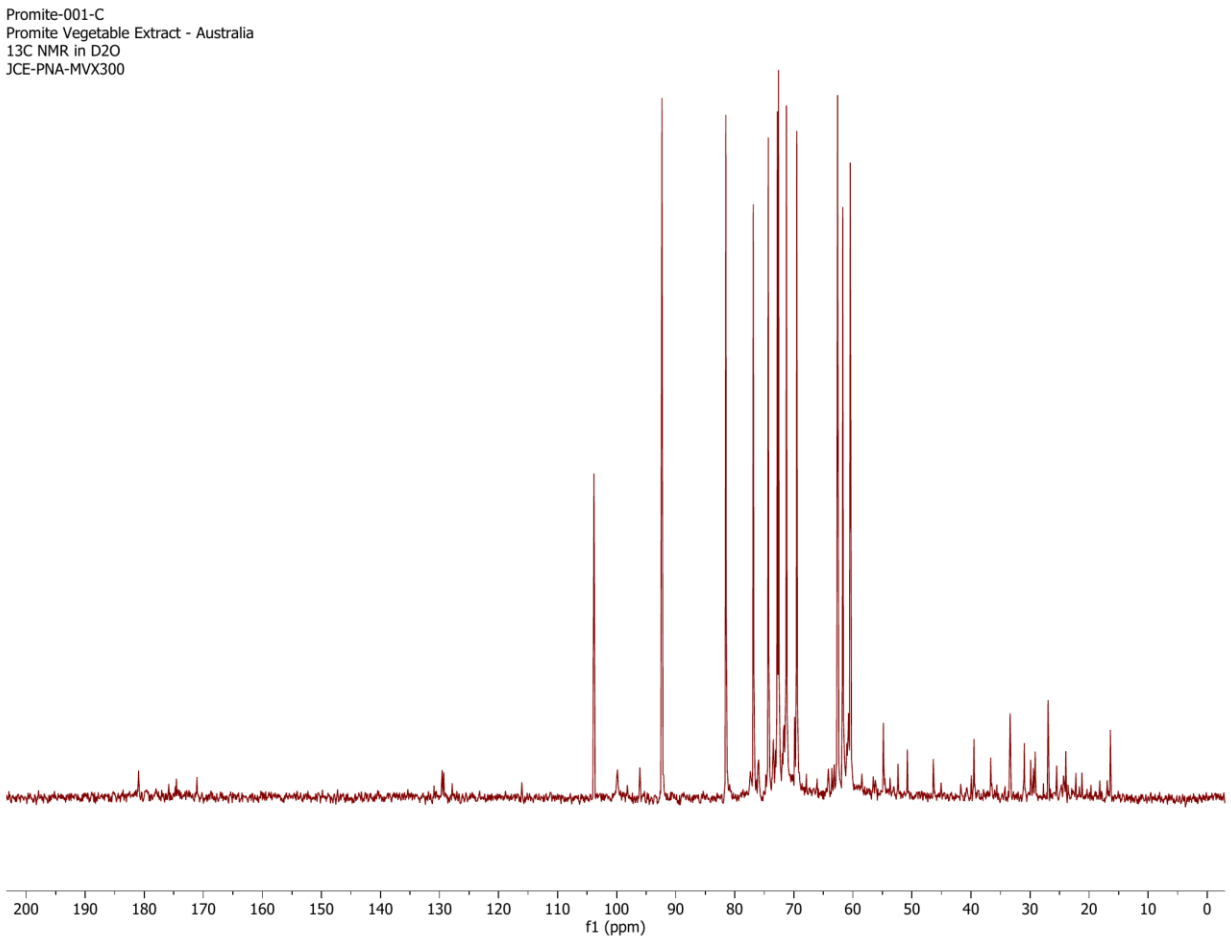


Figure 20. Proton-decoupled ^{13}C NMR spectrum (75 MHz, D_2O) of Promite; the axis reads 2.52 ppm below the DSS scale (sucrose C-2' at 103.83 ppm; Table 8). Note the absence of signals between 135 and 170 ppm.

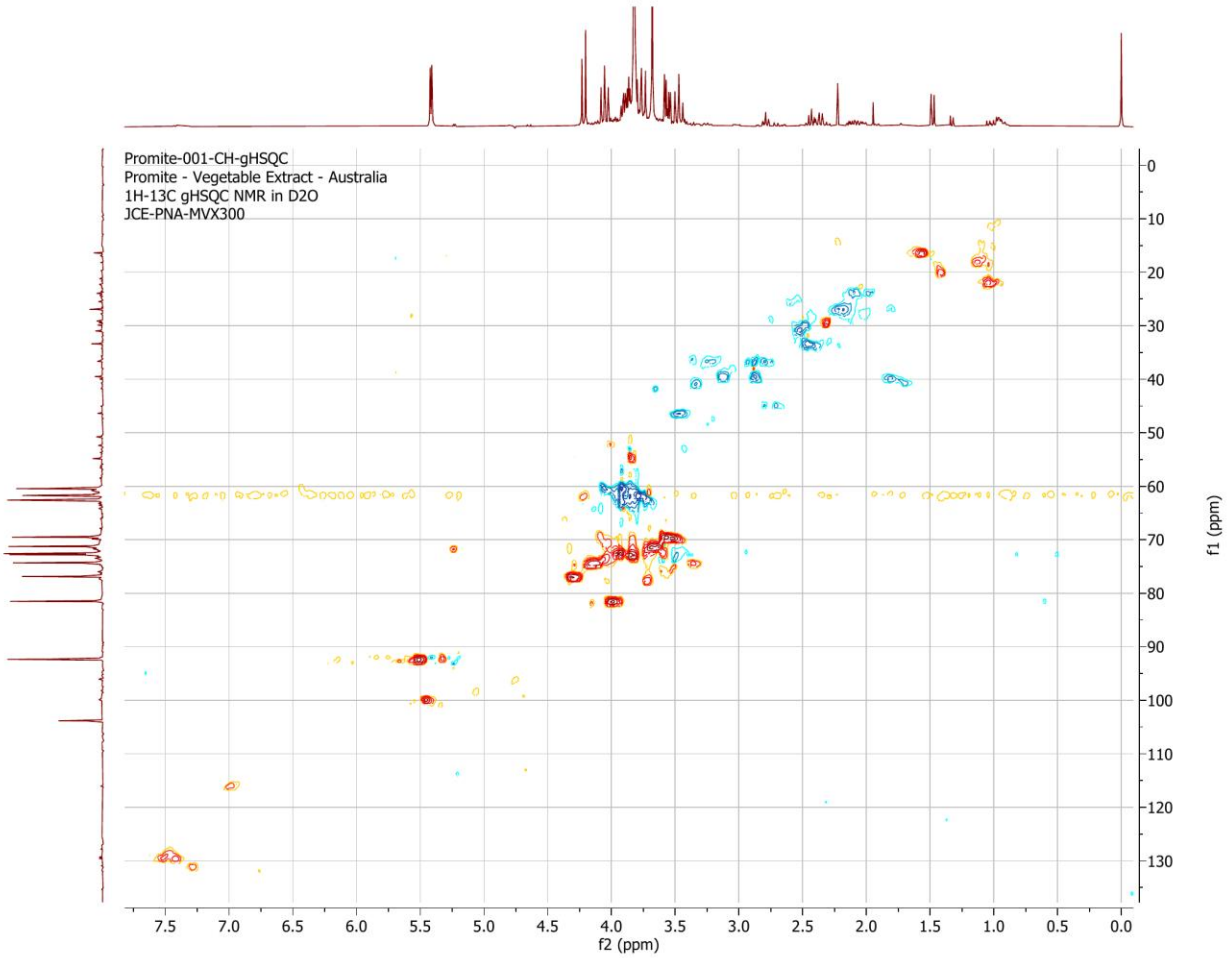


Figure 21. Multiplicity-edited ^{1}H–^{13}C gHSQC spectrum (300 MHz, D_2O) of Promite. The horizontal streak at δC 61 is t_1 noise from the intense sucrose CH_2 signals.

8.8 Comparative Feature Matrix

Table 6 condenses the observations of Sections 8.3 to 8.7 into a feature matrix, in which each product is scored on the relative prominence of the principal diagnostic signals. The scores are visual estimates from spectra scaled to their own tallest peak and are ordinal, not quantitative; they will be replaced by integral-based values once the raw data are processed against an internal standard. The scores are those of version 1.1 and have been left as they were; where the numerical analysis of Sections 8.10 to 8.15 leads to a different statement — for trehalose in the New Zealand product, the trimethylammonium compounds and the RNA-derived signals in particular — Tables 12 to 14 take precedence.

Table 6. Qualitative feature matrix for the five products (visual estimate from the ^{1}H spectra, supported by the ^{13}C and 2D data; +++ dominant, ++ prominent, + present, (+) weak or tentative, − not observed). The bottom row gives the inferred product class.

Feature (signal)	Marmite	Marmite XO	Vegemite	Sanitarium Marmite	Promite
Free amino acids (Ala 1.48 d; BCAA 0.9–1.05; α-CH envelope)	+++	+++	+++	++	+
Aromatic amino acids (Phe 7.3–7.4; Tyr 7.18/6.88; ^{13}C 116–135)	++	++	++	+	(+)

Feature (signal)	Marmite	Marmite XO	Vegemite	Sanitarium Marmite	Promite
Nucleobases / nucleosides / nucleotides (^{1}H 8.0–8.65, 5.9–6.15; ^{13}C 141–157, 87–89)	++	+++	+	(+)	–
Niacin H-2 (8.94 s)	(+)	(+)	(+)	(+)	–
Formate (8.46 s)	+	+	+	+	++
Choline-type N(CH$_3$)$_3$ (3.24 s; ^{13}C 54.5/68)	++	++	++	+	(+)
Succinate (2.41–2.47 s; ^{13}C 34.8)	++	++	++	+	(+)
Acetate (1.92–1.96 s; ^{13}C 24.0)	+	+	+	+	+
Lactate (1.33 d; HMBC 69/183)	+	+	++	+	+
Trehalose (5.19 d; C-1 94.0; symmetric HMBC)	+	(+)	+++	(+)	–
α-(1→4)/(1→6)-Glucan: dextrin, glycogen, maltodextrin (5.40 br; C-1 100.4; branch 4.95/98.5)	++	++	(+)	+	(+)
Sucrose (5.41 d, 4.22 d; C-2′ 104.5)	–	–	–	+++	+++
Free glucose (5.23 d / 4.65 d; C-1 92.9 / 96.7)	(+)	(+)	(+)	(+)	+
Citrate (2.53/2.66 AB; ^{13}C 46.3)	–	–	–	–	+
Levulinate (2.22 s / 29.6; 2.42 t; 2.75 t) — tentative	–	–	–	–	+
Olefinic CH=CH (HSQC 5.35/130) — tentative	–	–	(+)	–	–
Inferred class	Brewer's-yeast autolysate	Extended-maturation autolysate	Trehalose-rich brewer's-yeast autolysate	Yeast extract diluted with sucrose and maltodextrin	Acid-HVP and sucrose based spread with minor yeast extract

Read across, the matrix makes three points. The two British products and Vegemite share a common yeast-autolysate core — the same amino acid, organic acid, choline and RNA-derived inventory — and are separated from one another by the carbohydrate residue (α-glucan-rich in Burton, trehalose-rich in Melbourne) and by the depth of nucleolysis. The New Zealand product carries the same core but is diluted, in the analytical sense, by formulation carbohydrate to the point that its yeast-derived signals are secondary. Promite belongs to a different class in which the protein is of plant origin, the RNA degradation products are absent in every dimension examined (^{1}H, ^{13}C and HSQC), and the acid-hydrolysis markers are present. No conventional specification parameter in Table 3 would have delivered all three conclusions from a single measurement.

8.9 The United Kingdom Product against the Australasian Products: What Differs, and Why

The question that the comparison raises most sharply is why the Australasian products differ from the British one in exactly the way they do: why the two products from Australia and New Zealand are sweeter, why one carries a large trehalose signal and the other a large sucrose signal, and whether the sweetness is there for its own sake or to suppress bitterness. Table 7 sets the observed spectral differences beside the declared formulations and the manufacturing histories, and the paragraphs that follow argue the answer

from the chemistry. A distinction has to be drawn at the outset between an ingredient that has been added and a metabolite that has been retained, because the two carbohydrates in question fall on opposite sides of it.

Table 7. Contrast between the United Kingdom Marmite products and the Australasian products on NMR-observable features, declared formulation and inferred process.

Attribute	Marmite and Marmite XO (UK)	Vegemite (Australia)	Sanitarium Marmite (New Zealand)
Declared sweetener	None (sugars 1.2 g/100 g)	None (sugars 2.4 g/100 g; malt extract declared)	Sugar (sucrose) second ingredient; corn maltodextrin
Dominant carbohydrate signal	Branched α-(1→4)/(1→6)-glucan, H-1 5.35–5.40 ppm (2.3 residues per alanine in Marmite and 1.2 in XO, by ^{1}H); trehalose minor (0.29 and 0.08 mol per mol of alanine); glycerol prominent	Trehalose 5.19 d, C-1 96.0 ppm (DSS scale), symmetric HMBC; 1.2 mol per mol of alanine; α-glucan weak (2 residues per trehalose or fewer, against 9–19 in the UK products)	Sucrose (5.41, 4.22, 4.05, 3.67; C-2′ 106.4 ppm, DSS scale), about one-eighth inverted; trehalose 0.7 mol per mol of alanine (one molecule per ten of sucrose); α-glucan weak and branched as in the other yeast products, the declared maltodextrin not distinguishable within it
RNA-derived signals (^{1}H 7.85–8.35 and 5.9–6.15; ^{13}C 144–158, DSS scale)	Strong; strongest in XO. Mainly nucleosides (uridine, adenosine-type, guanosine-type); 5′-nucleotides not established; the 8.60 ppm singlet is histidine H-ε1	Weak (a single uridine-type H-1′/C-1′ response)	Weak (a single uridine-type H-1′/C-1′ response)
Free amino acid pattern	Ala dominant; BCAA and aromatic strong; Lys, Glu, Gly prominent	Ala dominant; Gly relatively strong; aromatics somewhat weaker	Same inventory, compressed by sucrose
Trimethylammonium singlets (3.21, 3.24, 3.27 ppm)	Strong; glycerophosphocholine, with free choline (0.3–0.4 of it) and betaine (0.6 of it in Marmite, 0.25 in XO)	Strong; glycerophosphocholine with free choline (0.3 of it); betaine not detected	Moderate; glycerophosphocholine with free choline (0.25 of it); betaine not detected
Succinate position (pH indicator)	2.47 (acidic)	2.41 in the ^{1}H spectrum of 2021 (near neutral); 2.46 in the gHSQC of the 2023 dissolution	2.47 (acidic)
Salt and salt substitute	Salt 10.8 g/100 g; no KCl declared	Sodium 3.3 g/100 g; mineral salt 508 (KCl) declared	Sodium ≈3.3 g/100 g; potassium chloride declared; potassium ≈2.8 g/100 g
Color agent	Vegetable juice concentrate; process color	Caramel 150c; malt extract	Caramel III
Inferred process signature	Long salt-induced autolysis and 7–28 day maturation: trehalose largely hydrolyzed (one-quarter as much in XO as in Marmite, relative to alanine), RNA degraded as far as the nucleosides, glucan retained as a branched dextrin	Autolysis regime that limits trehalase action (early enzyme kill and/or high-trehalose lager yeast); weak RNA-derived signals; little residual glucan	Trehalose-rich, glucan-poor yeast-extract base of the Vegemite type, then formulated with sucrose, maltodextrin, KCl, caramel and iron
Sensory consequence expected from the chemistry	Intense salty–umami–bitter profile; glutamate abundant, but synergy with 5′-nucleotides not demonstrated (the RNA products are present mainly as nucleosides)	Milder, slightly sweet (trehalose ≈45 % of sucrose sweetness); KCl off-note partly masked	Distinctly sweeter; sucrose suppresses bitterness and saltiness and masks KCl; described as "less tangy"

On the evidence available, trehalose was not added to Vegemite. Under the Australia New Zealand Food Standards Code an added ingredient must appear in the statement of ingredients, and trehalose, which is a permitted food ingredient in both jurisdictions, does not appear on the Vegemite label; the declared carbohydrate sources are the yeast extract itself and malt extract, and malt extract contributes maltose, maltotriose and glucose rather than trehalose.[21,22] The one loophole — that the components of a compound ingredient making up less than 5 percent of a food need not be itemized, so that a carrier-level quantity could in principle arrive inside a declared "flavor" — could not deliver trehalose as the dominant carbohydrate of the product, and it is in any case testable: commercial trehalose is manufactured enzymatically from maize starch, a C_4 carbon source, whereas the yeast's own trehalose is assembled from C_3 wort sugars, so that the $^{13}C/^{12}C$ ratio of trehalose isolated from the spread would distinguish the two origins by isotope-ratio mass spectrometry. The trehalose seen in Figure 9 is therefore taken to be the yeast's own reserve disaccharide, retained through the process, and the question becomes why the Melbourne process retains it and the Burton process does not. Two mechanisms are available and are not mutually exclusive. The first is the physiological state of the yeast at the point of extraction. Trehalose and glycogen are the two reserve carbohydrates of *Saccharomyces*; glycogen is laid down first as growth slows and is then mobilized in stationary phase and under carbon starvation, while trehalose is synthesized late and accumulates as a stress protectant, so that a cropped brewing yeast that has been held before processing progressively converts its glycogen into trehalose, the faster the warmer it is held.[37,73,74] Lager yeast harvested after cold maturation, or brewer's yeast stored for some days before extraction, will be trehalose-rich and glycogen-poor; freshly cropped yeast is the reverse. The Australian product's high trehalose and low α-glucan and the British product's high α-glucan and low trehalose are exactly the pattern that a difference in yeast condition at intake would produce, and the trehalose-to-glucan ratio measured by NMR is, in effect, a record of that condition. The second mechanism is the fate of the trehalases during extraction. *Saccharomyces* carries a cytosolic neutral trehalase and a vacuolar acid trehalase; both are active in the 30 to 50 °C range in which traditional salt-induced autolysis is conducted and over the days of a Marmite maturation, and both are inactivated by a heat shock above about 60 °C.[75] A process that begins with a rapid heat kill and then relies on added or heat-tolerant proteases, or that simply runs shorter and hotter, will retain trehalose that a long, mild, salt-driven autolysis consumes. The progressive loss of trehalose from Marmite to Marmite XO in Figures 1 and 3 is the within-brand demonstration of the same point. Which of the two mechanisms dominates in the Vegemite process cannot be decided from a single lot, but both are testable: the glycogen-to-trehalose relationship of the incoming yeast can be measured by the same ^1H method, and a time series through the autolysis would show whether trehalose falls.

Whether or not it was intended, the retained trehalose has sensory consequences. Trehalose is about 45 percent as sweet as sucrose, and it is non-reducing and therefore neither browns nor consumes free amino acids in Maillard chemistry during storage;[76,77] its use to suppress bitter, metallic and acetic off-notes in savory foods — including specifically the bitterness contributed by potassium salts — at inclusion levels of 1 to 2 percent is the subject of an industrial patent.[78] Vegemite declares potassium chloride (mineral salt 508) as a partial replacement for sodium chloride, and potassium chloride carries the bitter, metallic and chemical side-tastes that limit its use as a salt substitute; those side-tastes are reduced by sodium chloride and by sweeteners.[79,80] A product that is trehalose-rich by virtue of its process therefore arrives with a built-in partial mask for its own potassium chloride and for the peptide bitterness of the extract, and a manufacturer who found that a particular yeast supply or process setting gave a milder product would have every reason to keep it, without ever having "added" trehalose. That is the most economical reading of the Vegemite spectrum.

Sucrose, by contrast, is a deliberate addition in the New Zealand product and in Promite, declared as "sugar" and present at a level — second ingredient by weight in both, and 18 g of declared sugars per 100 g in Promite — that dominates the NMR spectra. Its purposes are the classical ones of formulation. Sweetness is the most effective single suppressor of bitterness in mixtures, and it also suppresses saltiness and sourness while being itself suppressed by them, so that adding sucrose to a salty, bitter, sour and umami base rounds the profile in all four directions at once; the phenomenon of mixture suppression is well characterized for

sucrose against bitter compounds and against sodium chloride.[80] Sodium chloride itself suppresses bitterness — which is one reason the British product, at 10.8 percent salt and with no sweetener, is palatable at all — and a product that replaces part of its sodium chloride with potassium chloride, as the New Zealand product does, loses part of that bitter suppression and gains the potassium off-notes, both of which sucrose repairs.[79,81] Sucrose additionally contributes body and gloss to the paste and, being non-reducing until it is inverted, does not itself darken the product, which is why caramel color is declared separately in the Australasian products. The historical record is consistent with this chemistry: the New Zealand recipe developed by Sanitarium in the 1930s and 1940s is described as having introduced sugar and caramel and as giving a milder, less tangy product than the British original, and Promite has been positioned since the 1950s as the sweeter alternative to Vegemite.[3,19,25] The NMR spectra add one further observation: in the New Zealand product the free glucose and fructose signals are small relative to the sucrose — the ^{13}C and ^{1}H intensities examined for version 1.2 correspond to the inversion of about one-eighth of it (Section 8.11) — so that the sucrose has not been substantially inverted, which indicates that it was added after the thermal steps of the process or that those steps are mild, and which makes the sucrose-to-invert ratio a candidate marker of thermal history for this product.

Two of the premises of this section are affected by the numerical analysis added in version 1.2 (Sections 8.10 to 8.15). The first is strengthened and extended. The ^{1}H spectra give a molar ratio of trehalose to free alanine of about 0.29 in Marmite, 0.08 in Marmite XO, 1.2 in Vegemite and 0.7 in the New Zealand Marmite, and a ratio of α-glucan residues to trehalose molecules of about 9 and 15 in the two British products against about 1 or less in the other two. The contrast between Burton and Melbourne on which the argument above rests is therefore real and large, and — contrary to the reading of version 1.1, in which the trehalose of the New Zealand product was taken for a trace beneath the sucrose — the Christchurch product lies between the two in trehalose relative to alanine and stands with Vegemite, not with the British Marmite, in its ratio of glucan to trehalose. An extract in which trehalose rivals or exceeds the soluble α-glucan is thus common to the two Australasian yeast products, so that whatever distinguishes the condition or the handling of the yeast in Melbourne from that in Burton appears to apply, at least in part, in Christchurch also; the observation is of one lot of each and decides nothing between the two mechanisms proposed above. The second premise is weakened. The RNA-derived material detected in the British products is present largely as nucleosides — uridine, an adenosine-type and a guanosine-type nucleoside — and the 8.6 ppm singlet formerly counted as a nucleotide signal is the H-ε1 of histidine. Nucleosides do not exhibit the synergy with glutamate that the 5′-nucleotides do, so that the inference formerly drawn from the stronger nucleobase signals to a more fully exploited umami synergy is not supported by the present data; it would require the demonstration of 5′-IMP or 5′-GMP, for which ^{31}P NMR is the appropriate experiment. The abundance of pyroglutamate, comparable with that of glutamate in both British products, points the same way, since it represents glutamine that was not converted to glutamate.

The answer to the question posed, then, is that it is both taste and bitterness reduction, and that the two are not separable: bitterness suppression is a taste effect, achieved in the Australasian products by sweetness (deliberate sucrose in New Zealand and in Promite, incidental trehalose in Vegemite) and in the British product by salt and by the umami of its glutamate-rich extract. The Australasian products achieve palatability by balancing rather than by intensifying. The deeper nucleolysis of the British product is visible in Table 7 as the stronger RNA-derived signals, but it does not follow, as was inferred in version 1.1, that the glutamate–5′-nucleotide synergy that underlies umami intensity is more fully exploited there, for the reason given in the preceding paragraph: the material identified is present largely as nucleosides, and the 5′-nucleotides themselves remain to be demonstrated.[45,46] Whether the milder Australasian profile was a deliberate design choice for a market that preferred it, an accident of yeast supply and process that was then retained, or a response to the introduction of potassium chloride, cannot be decided from composition alone. What the NMR data establish is the compositional mechanism by which each product achieves its balance, and they define the measurements — trehalose, α-glucan, sucrose and inversion products, nucleosides and 5′-nucleotides, glutamate and pyroglutamate, and the process ratios of Section 9.3 — that would allow a manufacturer to control it.

8.10 Numerical Recovery of the Two-Dimensional Data and Calibration of the Axes

The interpretation set out in Sections 8.3 to 8.9 was made from rasterized figures, and positions were therefore read to no better than a few tenths of a part per million in ^{13}C. For this version the processed data themselves were recovered. Five workspace files written by the PNA NMR Processor (version 2.6.4, November 11, 2026) were supplied, one per product, and each carries, unaltered, the MestReNova 14.1.1 document from which the figures had been printed. The processed traces that the workspaces hold for the two-dimensional experiments of Marmite XO, Vegemite and Promite are one-dimensional transforms of the two-dimensional time-domain data (262,144 points, unphased), and the two-dimensional experiments of Marmite and of the New Zealand Marmite do not appear among the spectra of their workspaces at all; none of these traces was used. The spectra as processed in MestReNova were taken instead: MestReNova stores each processed spectrum inside its document as a JPEG 2000 codestream (reversible wavelet transform, rate-limited to one-tenth of the uncompressed size) together with the intensity range and, for each dimension, the transmitter frequency, spectral width, reference offset and number of points, and these blocks were located and decoded to their full dynamic range. MestReNova therefore remains the processing engine of record for every number quoted below; no phasing, apodization, linear prediction or baseline correction was repeated, and the only operations applied here were peak picking and a constant correction of each axis.

The recovery was checked in three ways. The reference signal was found at 0.0000 ± 0.0001 ppm in each of the five one-dimensional ^{1}H spectra, which establishes that the axis records had been read correctly; the decoded matrices reproduce Figures 2, 6 to 8, 12, 13, 17, 18 and 21 feature for feature; and the compression error, estimated from the excursion of signal-free regions, is of the order of 10^{-5} of the full intensity range and lies below the thermal noise of every spectrum. Nine two-dimensional data sets were obtained in this way — gHSQC matrices for all five products, gHMBC matrices for Marmite XO, Vegemite and the New Zealand Marmite, and the 2D J-resolved matrix of Marmite XO — together with the one-dimensional ^{1}H, ^{13}C and DEPT spectra that accompany them. Their dimensions and acquisition parameters are collected in Table 8.

Cross-peaks were picked as local extrema of the lightly smoothed matrix and refined by parabolic interpolation. In the phase-sensitive gHSQC spectra a response was retained when it exceeded five times the noise standard deviation of the matrix and five times the local noise of its own row and column, the second condition being needed to reject the t_1 ridges that run through the most intense signals; responses lying on the truncation lobes of a signal at least six times stronger were set aside and are listed separately in the companion workbook. In the magnitude-mode gHMBC spectra, whose noise is not Gaussian, the threshold was set at 1.25 times the largest response found in a signal-free strip (δH 9.8 to 12.5). The numbers of cross-peaks retained are given in Table 8.

Two calibrations were then made. The ^{1}H axis of each two-dimensional spectrum was aligned to the referenced one-dimensional ^{1}H spectrum of the same product by cross-correlation of the F2 projection, region by region; the corrections range from −0.088 to +0.036 ppm and agree between spectral regions to about 0.02 ppm. The ^{13}C axes were placed on the DSS scale — the scale of the public metabolite databases — by matching resolved, pH-insensitive resonances (the alanine, leucine, valine and lysine side-chain carbons and the anomeric carbons of trehalose and sucrose) to the values deposited in the Biological Magnetic Resonance Data Bank, from which 132 metabolite entries recorded in D_2O at pH 7.4 and 298 K were retrieved for the purpose.[82,83] The corrections so obtained (Table 8) are large and are not uniform: +2.40 to +2.55 ppm for the five gHSQC spectra; +5.05, +2.85 and +4.85 ppm for the gHMBC spectra of Marmite XO, Vegemite and the New Zealand Marmite, the two largest belonging to the experiments whose parameter sets name dimethyl sulfoxide as the lock solvent; +3.11, −0.04, +2.91 and +2.52 ppm for the one-dimensional ^{13}C spectra of Marmite XO, Vegemite, the New Zealand Marmite and Promite; and +0.16 to +0.24 ppm for the DEPT sub-spectra. The statement in version 1.1 that the Marmite XO ^{13}C spectrum and the gHSQC F1 axes place the alanine methyl carbon at 17.0 ppm was a misreading of the figures: the numerical data place it at 15.75 ppm and at 16.3 to 16.4 ppm respectively, against 18.83 ppm on the DSS

scale. All ^{13}C positions in Sections 8.10 to 8.15 are quoted on the DSS scale after correction. The residual uncertainty is about ±0.02 ppm in ^{1}H, ±0.05 ppm for the one-dimensional ^{13}C spectra, ±0.3 to 0.4 ppm for the gHSQC F1 dimension (which rests on 64 to 256 t_1 increments extended by linear prediction) and ±0.5 to 1 ppm for the gHMBC F1 dimension, rising to about ±1.5 ppm for the 64-increment Vegemite experiment.

Four properties of the experiments bear on what follows. First, the gHSQC spectra are multiplicity-edited and tuned to a one-bond coupling of 146 Hz, so that CH and CH_3 groups appear with one sign and CH_2 groups with the other; the editing fails progressively as the true coupling departs from the set value and passes through a null at one and a half times that value, so that carbons with $^{1}J(C,H)$ of 195 to 220 Hz — the purine C-8 and C-2, the imidazolium C-ε1 of histidine and formate — give little or no response.[84] The absence of those cross-peaks is a property of the experiment and is not evidence that the compounds are absent. Second, the F1 window of the gHSQC is 170 ppm wide, and a carbon outside it is aliased; this is how formate is in fact observed. Third, the gHMBC spectra were acquired for a long-range coupling of 8 Hz and processed in magnitude mode, and they retain some one-bond responses as doublets separated by $^{1}J(C,H)$ in F2, which were recognized by reference to the gHSQC and excluded.[85] Fourth, none of the two-dimensional experiments is quantitative, and the size of the departure was measured. The gHSQC spectra were acquired with a relaxation delay of 1.0 s (a recycle time of about 1.2 s) and with the ^{12}C-bound-proton suppression element of the sequence switched on, so that the cross-peak volumes are weighted by ^{1}H longitudinal relaxation as well as by the size of the one-bond coupling; the one-dimensional ^{1}H spectra, by contrast, were acquired with 90° pulses and a recycle time of 8.3 s. When the ratio of the trehalose anomeric signal to the alanine methyl signal is taken from the two experiments, the gHSQC volume ratio exceeds the ^{1}H area ratio by factors of 2.2 in Marmite and 3.0 in Marmite XO and by one-fifth in Vegemite (for which the two experiments were run on different dissolutions), and agrees with it in the New Zealand Marmite. The N-methyl groups of the trimethylammonium compounds, taken as a third reference, are over-represented relative to alanine by factors of 1.3 to 2.0, and the trehalose signal relative to them by factors that range from 0.6 to 2.2, without regularity from product to product; and in the two sucrose-containing products the intense sucrose anomeric correlation is under-represented by 30 to 45 percent relative to that of alanine. The cause was not established — differential saturation at so short a recycle time is the most probable — but the practical conclusion does not depend on it. Ratios of like signals fare better: the ratio of the α-glucan to the trehalose anomeric signals agrees between the two experiments to within about one-third, the gHSQC favoring the polymer. For these reasons the statements of relative amount made below are taken from the ^{1}H spectra and from line heights in the ^{13}C spectra, cross-peak volumes being used only to compare signals of like kind, and every such figure is an estimate on a single lot. It should also be recorded that the ^{1}H spectra of Marmite and of Vegemite were acquired in March 2021, whereas the ^{13}C, DEPT and two-dimensional spectra of Vegemite were acquired in February 2023 on a fresh dissolution of the same lot; pH-dependent shifts need not agree between the two.

Table 8. Data sets recovered numerically from the MestReNova documents: processed matrix size, acquisition increments and transients, the constant corrections applied to the ^{1}H and ^{13}C axes (observed position plus correction equals corrected position; ^{13}C on the DSS scale) and the number of cross-peaks retained after rejection of artifacts.

Product and experiment (date acquired)	Processed matrix, F2 × F1 (points)	t_1 increments × transients	^{1}H axis correction (ppm)	^{13}C axis correction to DSS scale (ppm)	Cross-peaks retained
Marmite, gHSQC, multiplicity-edited (Mar 25 2021)	4096 × 2048	256 × 128	−0.031	+2.55	140
Marmite XO, gHSQC, multiplicity-edited (Feb 13 2023)	1024 × 1024	128 × 128	+0.036	+2.55	166
Marmite XO, gHMBC, magnitude mode (Feb 14 2023)	1024 × 1024	128 × 160	+0.001	+5.05	169
Marmite XO, 2D J-resolved, tilted and symmetrized (Feb 15 2023)	2048 × 128	128 × 16	+0.017	— (F1 ±110 Hz, 1.7 Hz per point)	72 (skyline projection)

Product and experiment (date acquired)	Processed matrix, F2 × F1 (points)	t_1 increments × transients	^{1}H axis correction (ppm)	^{13}C axis correction to DSS scale (ppm)	Cross-peaks retained
Vegemite, gHSQC, multiplicity-edited (Feb 14 2023)	8192 × 2048	64 × 128	−0.049	+2.40	74
Vegemite, gHMBC, magnitude mode (Feb 14 2023)	1024 × 512	64 × 160	+0.008	+2.85	52
New Zealand Marmite, gHSQC, multiplicity-edited (Feb 17 2023)	8192 × 4096	128 × 160	+0.009	+2.50	107
New Zealand Marmite, gHMBC, magnitude mode (Feb 17 2023)	1024 × 1024	200 × 200	+0.009	+4.85	63
Promite, gHSQC, multiplicity-edited (Feb 20 2023)	8192 × 2048	64 × 256	−0.088	+2.55	104
Marmite XO, ^{13}C{^{1}H} one-dimensional	262,144	—	—	+3.11	—
Vegemite, ^{13}C{^{1}H} one-dimensional	262,144	—	—	−0.04	—
New Zealand Marmite, ^{13}C{^{1}H} one-dimensional	262,144	—	—	+2.91	—
Promite, ^{13}C{^{1}H} one-dimensional	262,144	—	—	+2.52	—
Marmite XO, DEPT sub-spectra (CH_n, CH, CH_2, CH_3)	65,536	—	—	+0.21	—
Vegemite, DEPT sub-spectra (CH_n, CH, CH_2, CH_3)	65,536	—	—	+0.24	—
New Zealand Marmite, DEPT sub-spectra (CH_n, CH, CH_2, CH_3)	65,536	—	—	+0.16	—

8.11 Assignment of the gHSQC Spectra

Each retained cross-peak was compared with the one-bond correlations expected for the candidate constituents of a yeast autolysate, an acid-hydrolyzed vegetable protein and the declared formulation ingredients. A match required the same editing phase and agreement within 0.05 ppm in ^{1}H and 0.9 ppm in ^{13}C, with wider, one-sided windows for the carbons adjacent to a carboxyl group that titrates near the pH of these unbuffered solutions. Reference positions were taken numerically from the Biological Magnetic Resonance Data Bank wherever a usable entry exists; from the Human Metabolome Database for tyrosine (the data-bank entry was recorded at pH 11), the pyroglutamate anion, glycerophosphocholine and levulinate;[52] and from the random-coil compilation for residues bound in peptides.[67] The result is Table 9, in which the 155 assigned correlations are listed with the position found in each product, and Figures 22 to 28, in which the assignments are drawn on the spectra. A further check was available for four of the products in the one-dimensional ^{13}C spectrum, whose lines are located to ±0.05 ppm: the carbons of every constituent entered in Table 9 for Marmite XO, Vegemite, the New Zealand Marmite and Promite were sought there, and the positions found are tabulated in the companion workbook.

The free amino acids account for the greater part of the cross-peaks. Alanine, valine, leucine, isoleucine, threonine, serine, glycine, proline, lysine, arginine, glutamate, aspartate, methionine, phenylalanine and tyrosine are each identified in Marmite and in Marmite XO by every side-chain correlation that the experiment can deliver, and in the remaining products by most of them; only the α-carbons, which crowd into a band at δH 3.6 to 4.0 and δC 55 to 63, are incompletely resolved, the lysine, arginine, glutamate and glutamine α-CH signals forming a single envelope at 3.78/57.2. Methionine, which had been inferred in version 1.1 from a singlet at 2.14 ppm, is established by its S-methyl cross-peak at 2.13/16.8 in all five products and by the γ-methylene at 2.65/31.6. Asparagine (β-CH_2 2.93/37.3; α-CH 4.02/54.0) is present in the four yeast products and absent from Promite, in which neither correlation is found although aspartate is prominent; that is the pattern expected of acid hydrolysis, which deamidates asparagine and glutamine quantitatively. Glutamine is not established in any product: no methylene cross-peak is found at its γ position (2.45/33.5) in the yeast products, and the response at 2.43/33.3 in Promite belongs to levulinate

(below). Tryptophan is identified in the two British products alone, by three ring correlations in Marmite and five in Marmite XO (7.67/121.0, 7.48/114.5, 7.30/127.8, 7.22/124.7 and 7.13/121.9), and its absence from Promite is again as acid hydrolysis requires. Histidine is represented in the British products by a β-methylene pair at 3.35/28.7 and 3.28/29.1, positions that belong to the imidazolium form (the neutral-pH data-bank values are 3.13 to 3.22/30.7) and that are shared in part with tryptophan; its ring carbons are among those suppressed by the editing, and the confirmation comes from the gHMBC (Section 8.12).

Pyroglutamate proves to be a major constituent and not the minor one implied by its place in Table 5. Its α-CH (4.17/60.9), γ-CH_2 (2.40/32.3) and β-CH_2 (2.51 and 2.03/27.9) correlations are found in all four yeast products, and the γ-methylene cross-peak is as tall as that of glutamate in Marmite (signal-to-noise 34 against 35) and in Marmite XO (75 against 69), and about half as tall in Vegemite (16 against 28) and in the New Zealand product (42 against 78); in Promite only the α and β correlations are found, weakly. The corresponding carbons stand at 60.84, 32.33 and 27.88 ppm in the ^{13}C spectrum of Marmite XO, with a carboxylate at 182.67 ppm, and the ^{13}C line heights bear out the comparison: the three protonated carbons of pyroglutamate stand at 0.9 to 1.2 times the height of the corresponding carbons of glutamate in Marmite XO, and at 0.45 to 0.8 times in Vegemite and in the New Zealand Marmite. The data-bank entry for this compound (4.31/59.8 for the α position) does not agree with these values, whereas the Human Metabolome Database values for the anion do; the data-bank sample, 100 mM of the acid in 50 mM buffer, was in all likelihood incompletely neutralized. The assignment therefore rests on the internal consistency of three one-bond and two long-range correlations and on a database value that was not re-measured here, and a spike with authentic pyroglutamate is the proper confirmation. Chemically the finding is unsurprising — glutamine cyclizes to pyroglutamate on heating and on storage, and the simultaneous absence of glutamine and abundance of pyroglutamate is the signature of that conversion — but it matters for the interpretation of taste, because the cyclized product carries none of the umami of glutamate. 4-Aminobutyrate is indicated by a β-methylene at 1.90/26.3 and an α-methylene at 2.26 to 2.31/35.8 to 36.2 in the yeast products, and is confirmed in Marmite XO by long-range correlations.

A second population of amino acid signals lies beside the first, displaced to the random-coil positions of residues bound in peptides.[67] Internal alanyl residues give a methyl cross-peak at 1.41/19.4 in all five products; C-terminal alanyl residues one at 1.34/20.1, with the matching α-CH at 4.15/53.6, in the British products; threonyl residues a methyl at 1.21/21.7 in the four yeast products; valyl and isoleucyl residues methyls at 0.96/21.0 and 0.92/17.7, with the isoleucyl δ-methyl at 0.88/13.0 to 13.3 in Marmite and Promite and a leucyl δ-methyl at 0.88/23.6 in Marmite; aspartyl residues a β-methylene at 2.63 to 2.74/40.8 to 41.3 in the four yeast products, two parts per million downfield of that of free aspartate; lysyl residues a γ-methylene at 1.42/24.7; prolyl residues an α-CH at 4.45/63.3 and δ-methylenes at 3.63 to 3.80/50.3; and glycyl residues three separate methylene signals, at 3.92/43.3, 3.98/45.2 and 3.79/46.1, which are the positions of N-terminal, internal and C-terminal glycine. In Promite a leucyl β-methylene at 1.61/43.2 is the clearest of them. The gHSQC thus registers the incompleteness of the hydrolysis directly: the spreads contain small peptides in which most of the common residues are represented, alongside the free amino acids, and the ratio of the free to the bound signal of a given residue — alanine being the obvious choice, with its three resolved methyl positions — is a measure of the degree of hydrolysis that could be developed for the purposes of Section 9.3.

The carbohydrate region distinguishes the products most sharply, and the anomeric cross-peaks are compared in Figure 25. An α-(1→4)-linked glucan is present in all five (H-1/C-1 5.35 to 5.38/102.4 to 102.6; H-3/C-3 3.97/75.9; the 4-O-substituted C-4 at 3.65/80.1; H-2/C-2 3.63/74.3; H-5/C-5 3.83/74.1 to 74.4), at positions that reproduce those of the data-bank glycogen entry and of the starch and glycogen literature,[54,55] and it is accompanied in every case by the anomeric signal of α-(1→6)-linked branch residues at 4.97/101.1 and by signals of non-reducing terminal residues (H-3/C-3 3.71 to 3.75/75.5 to 75.6; C-4 at 72.0 ppm). The hydroxymethyl groups of the glucan, of trehalose and of the other hexoses are not resolved from one another, and their combined methylene response at 3.79 to 3.91/63.3 to 63.5 is the most intense cross-peak of the gHSQC spectra of both British products. The proportion of branch residues can be

estimated in two ways. As the ratio of the volume of the 4.97/101.1 cross-peak to the summed volume of the two anomeric cross-peaks — a comparison of like signals within one polymer — it is 0.200 ± 0.006 in Marmite, 0.216 ± 0.008 in Marmite XO, 0.23 ± 0.02 in Vegemite and 0.24 ± 0.05 in the New Zealand Marmite, against 0.14 ± 0.02 in Promite, the uncertainties being those of the noise alone. From the ^{1}H spectra, in which the two anomeric envelopes can be integrated for the British products, the same proportion is 0.13 in Marmite and 0.12 in Marmite XO. The two estimates err in opposite directions. The gHSQC favors the rapidly relaxing polymer over the small maltooligosaccharides that contribute sharper lines to the α-(1→4) envelope — the apex of the cross-peak lies at 5.35 to 5.38 ppm, upfield of the maximum of the ^{1}H envelope at 5.40 ppm, which is direct evidence of that bias — whereas the envelope integrals of the ^{1}H spectrum depend on where the baseline beneath a broad signal is drawn. The proportion therefore lies between about one residue in eight and one in five. Intact glycogen carries 7 to 12 percent of its residues at branch points and amylopectin 4 to 5 percent, whereas the limit dextrins that survive mashing and fermentation in beer are both short and heavily branched, and optimized heteronuclear NMR has been used to quantify precisely these structural classes in beer.[86,87] A branch content at or above the upper end of the glycogen range, together with the prominence of the terminal residues, points to a glucan that has been trimmed — the dextrin of the wort carried in with the brewery yeast, the limit dextrin left by the action of the yeast's own phosphorylase and glucosidases on its glycogen, or both — but the lower, ^{1}H-based estimate does not exclude intact glycogen, and the question is one for a measurement of molecular size (DOSY or size-exclusion chromatography) and not of linkage. The lower figure for Promite is in the direction expected of a glucose syrup derived from starch, although with the uncertainties attached it is separated from the British products by about three standard deviations only. The result bears also on the corn maltodextrin declared for the New Zealand Marmite. The branch proportion of that product is indistinguishable from that of the three yeast products to which no maltodextrin is added, and its ratio of glucan to trehalose is that of Vegemite (see below), so that the α-glucan observed is accounted for by the yeast extract itself: an added, sparsely branched maltodextrin is not distinguishable within it and, on the branch proportion, is unlikely to make up as much as half of it.

Cross-peaks of the yeast cell-wall mannan are found beside those of the glucan. Anomeric signals at 5.06 to 5.08/104.6 to 104.7 and 5.15 to 5.16/104.7 to 104.9 (terminal α-mannosyl residues), at 5.10 to 5.15/100.6 to 101.0 (2,6-di-O-substituted backbone residues) and, less securely, at 5.25/102.9 to 103.2 (2-O-substituted residues, which lie on the flank of the glucan signal), together with ring correlations at 4.10/72.8 (H-2/C-2 of unsubstituted residues) and 3.98 to 4.14/80.5 to 80.9 (the 2-O-substituted C-2), fall in the ranges established for the mannan of *Saccharomyces cerevisiae*.[88] They are clearest in Marmite and Marmite XO, weak in Vegemite and in the New Zealand product and absent from Promite, and they show that soluble mannoprotein is carried through the separation into the British spreads in observable quantity. Three weak responses of Marmite XO, each at a signal-to-noise ratio of 6 to 8, lie where a β-linked glucosyl residue is expected: at 4.54/105.4 (H-1/C-1), at 3.33 to 3.36/75.7 (H-2/C-2) and at 3.50 to 3.55/78.3 to 78.5 (H-3/C-3), against 4.49/105.4, 3.31/75.8 and 3.50/78.3 for the β-linked unit of gentiobiose in the data bank (the ring protons being paired with their carbons as in the experimental peak list of that entry). They may mark a fragment of cell-wall β-glucan and are recorded as tentative, since each is weak and neither a long-range correlation nor a ^{13}C anomeric line supports them. The last of the three had been read as free β-glucose; the ^{13}C spectrum of Marmite XO shows a line at 78.25 ppm but none at either anomeric position of free glucose, and free glucose is accordingly not entered for that product. Responses at 3.52/78.5 and 3.61/77.4 in Marmite, which are left unassigned, may have the same origin.

Trehalose is identified in all four yeast products by its H-1/C-1 correlation at 5.19 to 5.21/95.9 to 96.0 and by the ring correlations that can be resolved (all six in Vegemite), and it is absent from Promite. Its amount relative to the other constituents is best taken from the ^{1}H spectra, in which the anomeric doublet (two protons per molecule) and the alanine methyl doublet are both sharp. Each doublet was measured in two ways over the same limits, which were set 4 Hz beyond its outer lines: by fitting two Lorentzian lines on a straight local baseline, and by direct integration above a straight line drawn between the limits; both were repeated with the limits at 3 and at 5 Hz. The molar ratio of trehalose to free alanine obtained as the mean

of the two treatments is 0.29 in Marmite, 0.08 in Marmite XO, 1.25 in Vegemite and 0.68 in the New Zealand Marmite, and the six evaluations of each span about one-tenth of the value, or less, on either side (these and the other relative amounts quoted in this section are collected, with their ranges, in Table 13). A fit that allows the local baseline to curve gives values higher by one-tenth to one-third, but only by driving the fitted baseline below zero beneath the doublets of Vegemite and of the New Zealand product, and it was rejected. On the same basis the α-(1→4)-glucan envelope corresponds to 2.3 glucosyl residues per molecule of alanine in Marmite and 1.2 in Marmite XO; in Vegemite it is overlapped by the olefinic multiplet, so that 0.8 residue per alanine is an upper bound, and in the New Zealand product it is obtained only as the difference between the envelope and the sucrose doublet that stands upon it, about 0.8 residue per alanine with an uncertainty of a quarter of that figure. Expressed as glucosyl residues of the α-glucan per molecule of trehalose, the ^{1}H spectra give about 9 for Marmite and 15 for Marmite XO, against 0.6 at most for Vegemite and about 1.2 for the New Zealand Marmite; the gHSQC volumes of the anomeric cross-peaks, which compare like signals but favor the polymer, give 12, 19, 1.7 and 1.9 respectively. Three conclusions follow, and they do not depend on which measure is taken. Marmite XO carries between one-quarter and one-third of the trehalose and one-half of the α-glucan of standard Marmite relative to alanine. Alanine serves here only as a ruler, and since the free amino acids are themselves expected to increase when autolysis is prolonged, the two figures cannot be read as losses of that size; what is independent of the ruler is the ratio of glucan to trehalose, which is nearly twice as high in Marmite XO, so that the lower trehalose content of the matured product, surmised in version 1.1 from the appearance of the spectra, is confirmed to that extent, on one lot of each. Vegemite carries about four times as much trehalose as standard Marmite relative to alanine and a third or less of its glucan, a contrast of a factor of ten or more in the ratio of the two carbohydrates that no plausible difference in free alanine could produce. And the New Zealand Marmite, in which version 1.1 found only a trace of trehalose beneath the sucrose, carries more than twice as much trehalose as standard Marmite on the same basis, though only about half as much as Vegemite, and resembles Vegemite, not the British product, in its ratio of glucan to trehalose. In that product the sharp doublet near 5.2 ppm, left undecided in version 1.1, is resolved into trehalose (5.19/96.0) and the α-anomer of glucose (5.24/94.8); the ^{1}H spectrum gives one molecule of trehalose for ten of sucrose, and the ^{13}C spectrum, in which the trehalose C-1 line at 95.97 ppm stands at one-fifth of the height of the sucrose C-1 line at 94.87 ppm, gives the same figure.

Sucrose is assigned completely in the New Zealand Marmite and in Promite, all eleven protonated carbons being found at the data-bank positions, with the quaternary C-2′ at 106.36 ppm in the ^{13}C spectra. A weak response at 5.42/94.8 in Marmite suggests a trace of sucrose there also, plausibly from the declared vegetable juice concentrate, and is entered as tentative; the responses at 4.05/66.6 and 4.01/71.8 in the same spectrum would then be the accompanying fructose, although the former is also the position expected for the 5′-methylene of a 5′-nucleotide. Free glucose (α H-1/C-1 5.24/94.8; β H-1/C-1 4.66/98.6; β H-2/C-2 3.26/76.9; β H-3 and H-5 3.49/78.6) and free fructose (β-pyranose H-6/C-6 4.04/66.2, H-5/C-5 4.01/71.9 and, in the New Zealand Marmite, H-4/C-4 3.89/72.7; β-furanose H-3/C-3 4.11/78.4 there also; α-furanose H-5/C-5 4.06/84.1; with the quaternary anomeric carbon of the β-pyranose at 100.74 ppm) accompany the sucrose in both products. In the ^{13}C spectrum of the New Zealand Marmite the β-glucose C-1 and the fructopyranose C-2 lines, corrected for the anomeric and tautomeric proportions of the two sugars, amount to about 13 and 15 percent of the sucrose C-1 and C-2′ lines respectively; glucose and fructose are thus present in nearly equal amount, at roughly one-seventh of the sucrose, which is what the inversion of about one-eighth of the added sucrose in an acidic paste would produce. The ^{1}H spectrum supports the estimate: the α-anomeric doublet at 5.24 ppm, measured in the same way as the trehalose doublet, amounts to 7 percent of the sucrose H-1 doublet, which corresponds to reducing glucose at about 19 percent of the sucrose when the anomeric equilibrium is allowed for, the excess over the ^{13}C figure being attributable at least in part to the α reducing ends of oligosaccharides, which coincide with the α-anomer of free glucose in the ^{1}H spectrum. For Promite the ^{13}C lines give 6 percent for glucose and 4 percent for fructose, and the ^{1}H doublet reducing glucose at 13 percent of the sucrose; the inequality of the two sugars, and the larger ^{1}H figure, are as expected of a product that declares glucose syrup, and little of the sucrose of Promite need have been

inverted. The ^{13}C comparison is of peak heights of like carbons in spectra that were not acquired quantitatively, and an uncertainty of a third of each value should be allowed. A small doublet at 5.24 ppm (J = 3.8 Hz), about one-twentieth of the area of the trehalose doublet, is present also in the ^{1}H spectrum of Vegemite, where the α-anomeric proton of glucose would lie; but the ^{13}C spectrum of the later dissolution of that product shows no line at either anomeric position of glucose, at a signal-to-noise ratio below 2 where about 10 would be expected, and the doublet is left unassigned. Glycerol, finally, is far more prominent than version 1.1 recognized. Its methylene carbons at 65.18 ppm give the tallest line in the ^{13}C spectrum of Marmite XO — the line that had been assigned to the C-6 carbons of the glucan and trehalose, which lie at 63.2 ppm — with the methine carbon at 74.66 ppm at half the height, and the gHSQC shows the characteristic unequal methylene pair at 3.65 and 3.57/65.2 in Marmite and the same envelope in Marmite XO. Glycerol is found also in Vegemite; in the two sucrose-containing products its carbons coincide with C-6′ and C-5 of sucrose and cannot be assessed.

Among the organic acids, succinate gives an isolated methylene cross-peak at 2.46 to 2.48/35.4 to 35.8 in the four yeast products and is not found in the gHSQC of Promite, although a weak sharp singlet at 2.47 ppm in the ^{1}H spectrum of that product may be a trace of it; lactate is located by its methine at 4.13 to 4.15/71.1 to 71.2 in the British products and by its carbons at 71.2 and 22.9 ppm in the ^{13}C spectra of the others, its methyl signal being superposed on that of threonine at 1.33/22.5; and acetate gives a methyl cross-peak at 1.95 to 1.97/25.2 to 25.6 in Marmite XO, the New Zealand Marmite and Promite. The positions of the acetate and succinate signals are informative in themselves. Both lie downfield of the neutral-pH data-bank positions in ^{1}H (1.91 and 2.39 ppm) and upfield in ^{13}C (26.0 and 36.9 ppm), which is the direction and the size of displacement expected when a substantial fraction of each acid is protonated, and they place the apparent pH of the solutions used for the two-dimensional work close to 5;[89] the imidazolium form of histidine and the titration-shifted carbons of glutamate, lactate and citrate tell the same story. Every comparison with a neutral-pH database in this section carries that qualification, and it is the strongest single argument for the buffered re-acquisition proposed in Section 11.

In Promite two further acids are identified. Citrate, a declared ingredient, gives the AB methylene pair at 2.71 and 2.62/47.4 and a quaternary carbon at 78.46 ppm. Levulinate, entered as tentative in version 1.1 on the strength of a methyl singlet, is now supported by three one-bond correlations of comparable intensity — the methyl at 2.22/32.0 and two methylene groups at 2.78/42.0 and 2.43/33.3, the pattern of a CH_3–CO–CH_2–CH_2–COO^- chain — and by the three corresponding lines at 31.95, 42.00 and 33.49 ppm in the ^{13}C spectrum; none of the three is found in any yeast product. Acetone, the alternative considered earlier, has no methylene carbons and cannot account for the pattern. The ketone carbon was sought in the ^{13}C spectrum, which extends to 271 ppm and not to 200 ppm as the cropped figure suggested, and no line rises above 3.5 times the noise between 195 and 235 ppm; at the signal-to-noise ratio of the protonated carbons (12 to 24) a slowly relaxing, unprotonated carbonyl would not be expected to. The assignment is accordingly graded probable, and an authentic spike or a gHMBC acquired with an F1 window reaching 220 ppm would complete it. Formate, whose one-bond coupling of 195 Hz places it near the null of the editing and whose carbon at 173.9 ppm lies outside the F1 window, appears in the Promite gHSQC as an aliased response at δH 8.47 and an apparent δC of 4.1, which unfolds to 174.1 ppm; no such response is detectable in the yeast products, in which formate is observed in the ^{1}H spectra only.

The trimethylammonium singlet of the yeast products, attributed in version 1.1 to choline and glycerophosphocholine jointly, is resolved into its components. The principal N-methyl cross-peak at 3.22 to 3.23/56.7 to 56.8 is accompanied in Marmite and Marmite XO by an N-methylene at 3.67/68.6 and an O-methylene at 4.32/62.2, in Marmite and the New Zealand product by a methylene at 3.89 to 3.95/69.3 to 69.7, and in Marmite XO by a methine at 3.91/73.1, the position of the glycerol CH of the same compound; in the ^{13}C spectrum of Marmite XO the last appears as a doublet at 69.23 and 69.15 ppm, a splitting of 6 Hz that is the two-bond coupling to phosphorus of a CH_2–O–P group. These are the correlations of glycerophosphocholine, whose ^{1}H shifts are well established,[90] and not those of free choline, whose N-methylene and hydroxymethylene carbons lie at 70.1 and 58.3 ppm. Free choline is nevertheless present as

a lesser species: the ^{1}H spectra of all four yeast products show a second sharp N-methyl singlet 0.03 ppm upfield of the first (3.21 against 3.24 ppm in Marmite XO), whose fitted area is between one-quarter and two-fifths of that of the principal singlet, and the gHMBC of Marmite XO connects it to a carbon at 70.2 ppm. A third sharp singlet, 0.035 ppm downfield of the principal one (3.27 ppm), is found in the two British products only, at about 0.6 of the area of the glycerophosphocholine singlet in Marmite and about one-quarter of it in Marmite XO. It was not listed by the automatic peak picking, in which it is merged with the much stronger response beside it, but the matrices show it plainly. In the gHSQC of Marmite, which was acquired with twice as many increments as that of Marmite XO and is digitized four times more finely in F2, it forms a separate maximum at 3.26/56.1 to 56.2, displaced from the glycerophosphocholine cross-peak (3.22/56.7) in both dimensions; in the more coarsely digitized matrix of Marmite XO it forms a shoulder whose carbon maximum lies at 56.3 ppm, against 56.7 ppm for the principal signal; and in the gHMBC of Marmite XO the column at its frequency shows the same displaced N-methyl carbon and a correlation to a methylene carbon at 68.8 to 69.0 ppm that stands as a maximum separate from that of glycerophosphocholine (68.6 ppm) and from that of choline (70.2 ppm). The DEPT methyl sub-spectrum of Marmite XO shows a line at 56.03 ppm beside those at 56.64 and 56.52 ppm, and the ^{13}C spectrum weak lines at 68.79 and 172.02 ppm. These are the shifts of glycine betaine, for which the data bank gives 3.25/56.0 for the N-methyl groups, 3.89/68.8 for the methylene group and 172.0 ppm for the carboxylate, and the compound is entered as confirmed for Marmite XO and probable for Marmite, with the reservation that its methylene protons, which fall in the most crowded part of the spectrum, and their correlation to the carboxylate carbon were not observed. Version 1.1 had excluded betaine on the absence of exactly those signals. In the ^{1}H spectra of Vegemite and of the New Zealand Marmite a weak line is found at 3.271 ppm, about one-tenth of the height of the glycerophosphocholine singlet, among multiplet lines of other constituents (the β-protons of phenylalanine and the δ-protons of arginine fall there, and in the New Zealand product H-2 of β-glucose also); the gHSQC of Vegemite shows nothing at its frequency, and that of the New Zealand product only a response of one-twentieth of the glycerophosphocholine cross-peak, which is at the level of the lobes that surround that peak. Betaine, if present in those two products, is therefore below about one-fifth of the glycerophosphocholine, and more probably below one-tenth. Phosphocholine cannot be assessed: its N-methyl singlet would coincide with that of glycerophosphocholine, and its O-methylene, expected at 4.16/60.9, with the α-CH of pyroglutamate, which has the opposite sign in the edited spectrum. Promite shows no trimethylammonium cross-peak above the picking threshold; the integral over the N-methyl region of its gHSQC is positive at about four standard deviations and, relative to the alanine cross-peak, amounts to no more than one-tenth of the value found in the yeast products, so that a trace is not excluded. The origin of the betaine cannot be decided from these data. Cereal grains are among the richest dietary sources of the compound, and beet is the classical one,[91] so that it may arrive with the wort carried in by the brewery yeast, with the vegetable juice concentrate that is declared for the British product and not for the Australasian ones, or with a yeast grown on beet molasses; the bearing of the observation on the use of betaine as a marker of yeast source is taken up in Section 10.4.

The products of RNA degradation appear in the gHSQC through their ribose carbons, the ring CH groups of the purines being suppressed by the editing. Uridine is identified in Marmite XO by six correlations — H-6/C-6 7.85/144.6, H-5/C-5 5.90/105.1, H-1′/C-1′ 5.90/91.8, H-4′/C-4′ 4.14/87.0, H-2′/C-2′ 4.35/76.2 and H-3′/C-3′ 4.23/72.2 — and in Marmite by two; a purine nucleoside of the adenosine type by H-1′/C-1′ 6.04/91.0, H-4′/C-4′ 4.28/88.2, H-2′/C-2′ 4.75/76.5 and H-3′/C-3′ 4.42/73.2; and a guanosine-type ribose by H-1′/C-1′ 5.88/90.4. The anomeric positions are those of the nucleosides: the 5′-nucleotides have C-1′ near 89.6 to 90.1 ppm with H-1′ at 6.1 ppm and, more decisively, a 5′-methylene carbon at 66 ppm, and no methylene cross-peak is found at 4.03/66.2 in Marmite XO. A weak methine at 4.39/87.4 in both British products is in the position of C-4′ of a 5′-nucleotide and is entered as tentative. No cytidine, cytosine or uracil correlation is found. In Vegemite and the New Zealand product a single weak uridine-type H-1′/C-1′ response is all that is detected, and in Promite none. What the gHSQC shows, therefore, is that the RNA-derived material of the British products is present largely as nucleosides. That is the outcome expected of

the endogenous ribonuclease and phosphatase route of Section 4.3, and it bears directly on the argument of Section 8.9, since the nucleosides do not share the flavor activity of the 5′-nucleotides.

Three further observations complete the inventory. The weak olefinic response of Vegemite, flagged as tentative in version 1.1, is found at 5.31/132.0 and is accompanied, as was predicted there, by an allylic methylene at 2.02/29.8 and by the methylene chain signal at 1.32/31.9, the latter among the stronger methylene cross-peaks of the spectrum; no bis-allylic methylene is present. These are the signals of mobile monounsaturated fatty acyl chains, which is the only kind of unsaturation that *Saccharomyces* synthesizes, and they are peculiar to Vegemite among the five products. Ethanol (1.18/19.8 and 3.66/59.9, with ^{13}C lines at 19.5 and 60.0 ppm) is identified in the New Zealand Marmite, where the sharp methyl triplet also generates a conspicuous t_1 ridge. And a methyl doublet at 1.14 ppm on a carbon at 20.7 ppm in Marmite XO, which the gHMBC connects to an oxygenated carbon at 74.1 ppm, is tentatively assigned to 2,3-butanediol, a familiar minor product of brewery fermentation; the carbinol carbon is too far downfield for lactate, threonine, 1,2-propanediol or 3-hydroxybutyrate.

Table 9. One-bond ^{1}H–^{13}C correlations assigned in the multiplicity-edited gHSQC spectra of the five products. Entries are δH/δC in ppm after correction of the axes (^{13}C on the DSS scale); two δH values are given where the protons of a methylene group are resolved. Reference positions are from the Biological Magnetic Resonance Data Bank (B), the Human Metabolome Database (H), random-coil values for peptide residues (R) or the literature cited in the text (L). An entry of the form (^{13}C x) indicates that no cross-peak was retained but that the carbon was found at x ppm in the one-dimensional ^{13}C spectrum; –, not observed.

Constituent and group	Reference δH/δC (source)	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Free amino acids						
Alanine, β-CH_3	1.47/18.8 (B)	1.49/18.9	1.49/18.9	1.48/18.8	1.48/18.9	1.48/18.9
Alanine, α-CH	3.77/53.2 (B)	3.79/53.3	3.83/53.3	3.80/53.4	3.81/53.4	3.76/53.9
Valine, γ-CH_3	1.03/20.7 (B)	1.05/20.6	1.05/20.6	1.05/20.5	1.04/20.7	1.04/20.6
Valine, γ′-CH_3	0.98/19.4 (B)	1.00/19.5	1.01/19.6	(^{13}C 19.44)	1.00/19.6	(^{13}C 19.44)
Valine, β-CH	2.27/31.8 (B)	–	2.28/31.4	(^{13}C 31.71)	(^{13}C 31.72)	2.22/32.0
Valine + threonine, α-CH (unresolved pair)	3.60/63.1 (B)	3.61/63.1	3.64/63.0	3.62/63.0	(^{13}C 63.15)	3.62/63.7
Leucine, δ-CH_3	0.96/24.8 (B)	0.97/24.7	0.96/24.6	0.96/24.4	0.96/24.8	0.95/24.4
Leucine, γ-CH	1.70/26.9 (B)	1.74/27.0	1.73/27.0	(^{13}C 26.87)	(^{13}C 26.89)	(^{13}C 26.87)
Leucine, β-CH_2	1.70/42.5 (B)	1.73/42.5	1.72/42.5	1.73/42.4	1.71/42.5	1.72/42.3
Leucine, α-CH	3.72/56.1 (B)	–	3.74/56.1	(^{13}C 56.21)	(^{13}C 56.25)	(^{13}C 56.22)
Isoleucine, δ-CH_3	0.93/13.8 (B)	0.96/13.6	0.95/13.7	0.92/13.5	0.91/13.7	0.93/13.9
Isoleucine, γ′-CH_3	1.00/17.4 (B)	1.01/17.4	1.01/17.4	(^{13}C 17.41)	1.00/17.4	1.01/17.3
Isoleucine, γ-CH_2	1.46, 1.25/27.2 (B)	1.49, 1.24/27.2	1.49, 1.25/27.0	(^{13}C 27.18)	1.51/27.1	(^{13}C 27.19)
Isoleucine, β-CH	1.97/38.6 (B)	1.95/38.7	1.98/38.6	(^{13}C 38.52)	1.98/38.6	–
Isoleucine, α-CH	3.66/62.2 (B)	3.68/62.3	3.69/62.2	3.69/62.1	(^{13}C 62.14)	(^{13}C 62.27)
Threonine, γ-CH_3 (with lactate CH_3)	1.32/22.2 (B)	1.34/22.6	1.34/22.3	1.33/22.5	1.33/22.7	1.32/22.6
Threonine, β-CH	4.25/68.7 (B)	4.27/68.6	4.28/68.5	4.28/69.3	(^{13}C 68.74)	4.28/68.6
Serine, β-CH_2	3.95/62.9 (B)	3.98/62.9	4.00/62.9	3.98/63.0	3.98/62.9	3.96/62.9
Serine, α-CH	3.83/59.1 (B)	3.87/59.1	3.88/59.0	3.88/59.3	(^{13}C 59.09)	(^{13}C 59.07)
Glycine, α-CH_2	3.55/44.2 (B)	3.57/44.2	3.59/44.3	3.58/44.3	3.57/44.3	3.56/44.3
Proline, α-CH	4.12/63.9 (B)	4.14/63.9	4.15/63.8	(^{13}C 63.98)	(^{13}C 63.94)	4.13/64.5
Proline, δ-CH_2	3.41, 3.33/48.8 (B)	3.43, 3.38/48.9	3.39/48.9	(^{13}C 48.88)	(^{13}C 48.95)	3.38/48.9
Proline, β-CH_2	2.34, 2.06/31.7 (B)	2.06/31.7	2.03/31.6	(^{13}C 31.71)	2.39, 2.08/32.0	2.33/31.1
Proline, γ-CH_2	2.00/26.5 (B)	–	1.99/26.7	(^{13}C 26.45)	1.97/26.8	2.00/26.2
Lysine, ε-CH_2	3.02/41.8 (B)	3.03/41.9	3.02/41.9	3.03/42.0	3.03/42.0	3.03/42.0
Lysine, δ-CH_2	1.72/29.1 (B)	1.72/29.0	1.71/29.0	1.73/29.1	1.71/29.0	1.71/29.1

Constituent and group	Reference δH/δC (source)	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Lysine, γ-CH_2	1.47/24.1 (B)	1.43/24.7	1.49/24.2	1.47/24.6	–	–
Lysine, β-CH_2	1.90/32.6 (B)	1.91/32.6	1.92/32.5	1.90/32.0	1.90/32.5	(^{13}C 32.41)
Arginine, δ-CH_2	3.24/43.2 (B)	3.22/43.3	3.25/43.3	3.25/43.3	3.25/43.3	3.25/43.3
Arginine, β-CH_2	1.91/30.3 (B)	1.92/30.3	1.96/30.3	(^{13}C 30.26)	1.94/30.3	1.94/30.3
Arginine, γ-CH_2	1.68/26.6 (B)	1.66/26.7	1.68/26.5	(^{13}C 26.65)	(^{13}C 26.70)	(^{13}C 26.46)
Lysine, arginine, glutamate, glutamine, α-CH (unresolved envelope)	3.75/57.2 (B)	3.78/57.2	3.79/57.2	3.78/57.2	(^{13}C 57.13)	3.76/57.2
Glutamate, γ-CH_2	2.34/36.2 (B)	2.38/36.0	2.39/35.9	2.37/36.0	2.38/35.9	2.37/35.8
Glutamate, β-CH_2	2.08/29.7 (B)	2.10/29.6	2.08/29.5	2.12/29.6	2.10/29.5	2.09/29.5
Aspartate, β-CH_2	2.80, 2.67/39.2 (B)	2.80/39.2	2.80/39.1	2.79/39.3	2.79/39.0	2.79, 2.65/39.4
Aspartate, α-CH	3.89/54.9 (B)	3.91/54.9	3.92/54.8	3.90/55.2	(^{13}C 54.90)	3.92/54.7
Asparagine, β-CH_2	2.94, 2.84/37.3 (B)	2.92/37.3	2.95/37.2	2.94/37.3	2.93/37.3	–
Asparagine, α-CH	3.99/54.0 (B)	4.02/54.0	4.03/54.0	4.02/54.1	4.02/54.0	–
Methionine, S-CH_3	2.12/16.6 (B)	2.13/16.8	2.14/16.8	2.13/16.8	2.13/16.8	2.13/16.9
Methionine, γ-CH_2	2.63/31.5 (B)	2.65/31.7	2.65/31.6	(^{13}C 31.71)	2.64/31.7	2.65/31.3
Phenylalanine, H-3/5	7.42/131.8 (B)	7.40/131.7	7.38/131.7	7.39/131.4	7.41/131.8	7.42/131.9
Phenylalanine, H-4	7.37/130.4 (B)	7.37/130.3	7.35/130.3	(^{13}C 130.33)	7.37/130.5	(^{13}C 130.36)
Phenylalanine, H-2/6	7.32/132.1 (B)	7.33/131.9	7.33/132.0	7.33/131.9	7.33/132.0	7.33/132.1
Phenylalanine, β-CH_2	3.27, 3.12/39.1 (B)	3.27, 3.14/39.0	3.27, 3.13/38.9	3.14/39.0	3.13/39.0	3.27, 3.11/39.0
Phenylalanine + tyrosine, α-CH	3.98/58.7 (B)	4.00/58.7	4.01/58.7	3.99/58.7	(^{13}C 58.76)	(^{13}C 58.73)
Tyrosine, H-2/6	7.18/133.5 (H)	7.19/133.3	7.16/133.2	7.19/133.3	7.18/133.4	7.20/133.7
Tyrosine, H-3/5	6.89/118.6 (H)	6.89/118.5	6.87/118.4	6.88/118.5	6.89/118.6	6.91/118.5
Tyrosine, β-CH_2	3.19, 3.05/38.3 (H)	3.07/38.3	(^{13}C 38.39)	(^{13}C 38.14)	(^{13}C 38.16)	(^{13}C 38.14)
Tryptophan, H-4	7.72/121.2 (B)	7.70/121.1	7.67/121.0	–	–	–
Tryptophan, H-7	7.53/114.7 (B)	7.49/114.6	7.48/114.5	–	–	–
Tryptophan, H-2	7.31/127.8 (B)	–	7.30/127.8	–	–	–
Tryptophan, H-6	7.27/124.8 (B)	7.24/124.7	7.22/124.7	–	–	–
Tryptophan, H-5	7.19/122.1 (B)	–	7.13/121.9	–	–	–
Histidine (imidazolium form), β-CH_2 (with tryptophan β-CH_2)	3.33, 3.25/28.9 (B)	3.34, 3.27/29.0	3.35, 3.28/28.9	–	–	–
4-Aminobutyrate (GABA), α-CH_2	2.29/36.6 (B)	2.27/35.9	2.31/35.9	2.25/36.2	2.26/35.8	–
4-Aminobutyrate (GABA), β-CH_2	1.89/26.4 (B)	1.91/26.2	1.91/26.2	–	1.89/26.3	1.90/26.4
Pyroglutamate (anion), α-CH	4.17/60.9 (H)	4.18/61.0	4.17/60.9	4.18/61.6	4.17/60.9	4.15/61.4
Pyroglutamate (anion), γ-CH_2	2.40/32.3 (H)	2.39/32.3	2.40/32.3	2.41/32.3	2.39/32.3	–
Pyroglutamate (anion), β-CH_2	2.50, 2.03/27.9 (H)	2.50, 2.05/27.9	2.51, 2.03/27.8	2.50/27.9	2.51, 2.06/27.9	2.50/28.2
Peptide-bound residues (random-coil positions)						
Alanyl residue, β-CH_3 (internal)	1.40/19.3 (R)	1.42/19.3	1.41/19.4	1.41/19.4	1.40/19.4	1.40/19.1
Alanyl residue, β-CH_3 (C-terminal)	1.33/20.1 (B)	1.34/20.2	1.34/20.1	–	–	–
Alanyl residue, α-CH (C-terminal)	4.12/53.7 (B)	–	4.15/53.6	–	–	–
Threonyl residue, γ-CH_3	1.21/21.6 (R)	1.22/21.6	1.21/21.7	1.19/21.6	1.20/21.7	–
Valyl residue, γ-CH_3	0.95/21.0 (R)	0.97/20.9	0.95/21.2	–	0.92/20.7	0.95/21.0
Valyl residue, β-CH	2.10/32.9 (R)	2.11/32.9	2.08/32.9	–	–	–
Isoleucyl residue, γ′-CH_3	0.92/17.6 (R)	0.91/17.6	0.93/17.7	–	0.91/17.7	0.92/17.7
Leucyl residue, β-CH_2	1.62/43.0 (R)	–	–	–	–	1.61/43.2
Lysyl residue, γ-CH_2	1.42/24.7 (R)	–	1.42/24.7	1.41/24.9	1.40/24.6	–
Prolyl residue, α-CH	4.44/63.3 (R)	4.47/63.4	4.45/63.3	–	–	–

Constituent and group	Reference δH/δC (source)	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Prolyl residue, δ-CH_2	3.80, 3.64/50.4 (R)	3.80, 3.62/50.5	3.63/50.2	–	–	–
Leucyl residue, δ-CH_3	0.90/23.3 (R)	0.88/23.6	(^{13}C 23.34)	–	–	–
Isoleucyl residue, δ-CH_3	0.86/12.9 (R)	0.88/13.0	(^{13}C 12.75)	–	–	0.88/13.3
Aspartyl residue, β-CH_2	2.72, 2.65/41.1 (R)	2.74, 2.69/41.1	2.74/40.8	2.64/41.3	2.72, 2.63/40.9	–
Glycyl residue, α-CH_2 (internal)	3.97/45.2 (R)	3.98/45.3	3.98/45.2	–	–	–
Glycyl residue, α-CH_2 (C-terminal)	3.77/46.1 (B)	3.78/46.1	3.79/46.1	3.79/46.4	–	–
Glycyl residue, α-CH_2 (N-terminal)	3.90/43.3 (R)	3.91/43.3	3.92/43.3	–	–	–
Internal residues (Lys, Glu, Gln, Arg, Met, Leu), α-CH envelope	4.36/56.0 (R)	4.35/56.1	4.39/55.9	4.33/56.3	–	–
C-terminal / N-acylated residue, α-CH	4.18/57.5	4.18/57.5	4.18/57.6	4.18/57.5	–	–
Trimethylammonium compounds						
Glycerophosphocholine, $N(CH_3)_3$	3.22/56.7 (H)	3.22/56.7	3.23/56.7	3.23/56.8	3.22/56.8	–
Glycerophosphocholine, N-CH_2	3.67/68.7 (H)	3.67/68.6	3.68/68.5	–	3.66/68.8	–
Glycerophosphocholine, O-CH_2 (choline)	4.32/62.2 (H)	4.32/62.2	4.33/62.2	–	–	–
Glycerophosphocholine, glycerol CH_2OP	3.94, 3.87/69.3 (H)	3.95/69.7	–	–	3.89/69.3	–
Glycerophosphocholine, glycerol CHOH (may include mannan H-3/C-3)	3.90/73.4 (H)	–	3.91/73.1	–	–	–
Betaine, $N(CH_3)_3$	3.25/56.0 (B)	3.26/56.2	3.27/56.3	–	–	–
Organic acids						
Lactate, CH	4.10/71.2 (B)	4.13/71.2	4.15/71.1	–	–	–
Acetate, CH_3	1.91/26.0 (B)	–	1.97/25.5	–	1.95/25.6	1.95/25.2
Succinate, CH_2	2.39/36.9 (B)	2.48/35.4	2.48/35.4	2.46/35.8	2.46/35.6	–
Citrate, CH_2 (AB)	2.67, 2.53/48.4 (B)	–	–	–	–	2.71, 2.62/47.4
Levulinate (4-oxopentanoate), CH_3	2.22/31.9 (H)	–	–	–	–	2.22/32.0
Levulinate (4-oxopentanoate), C-3 H_2 ($CH_2C{=}O$)	2.76/41.8 (H)	–	–	–	–	2.78/42.0
Levulinate (4-oxopentanoate), C-2 H_2 (CH_2COO^-)	2.40/33.2 (H)	–	–	–	–	2.43/33.3
Formate, CH (F1-aliased response; δC after unfolding)	8.45/173.9 (B)	–	–	–	–	8.47/174.1
Carbohydrates and polyols						
Glycerol, CH_2OH	3.65, 3.56/65.2 (B)	3.64, 3.57/65.2	3.65/65.2	3.69/65.1	3.58/65.4	–
Glycerol, CHOH	3.77/74.8 (B)	3.79/75.0	–	3.73/75.6	–	–
α,α-Trehalose, H-1/C-1	5.19/95.9 (B)	5.19/95.9	5.20/95.9	5.21/96.0	5.19/96.0	–
α,α-Trehalose, H-2/C-2	3.64/73.8 (B)	–	–	3.67/74.0	3.66/73.9	–
α,α-Trehalose, H-3/C-3	3.84/75.2 (B)	–	–	3.84/74.9	–	–
α,α-Trehalose, H-4/C-4	3.44/72.4 (B)	3.44/72.2	3.46/72.2	3.46/72.4	3.46/72.1	–
α,α-Trehalose, H-5/C-5	3.82/74.9 (B)	–	–	3.84/74.9	–	–
α,α-Trehalose, H-6/C-6	3.86, 3.76/63.2 (B)	–	–	3.85, 3.79/63.4	–	–
Sucrose, Glc H-1/C-1	5.40/94.9 (B)	5.42/94.8	–	–	5.42/94.9	5.42/94.9
Sucrose, Glc H-2/C-2	3.55/73.8 (B)	–	–	–	3.56/73.8	3.55/73.9
Sucrose, Glc H-3/C-3	3.75/75.3 (B)	–	–	–	3.75/75.4	3.76/75.3
Sucrose, Glc H-4/C-4	3.46/71.9 (B)	–	–	–	3.46/72.1	3.48/72.0
Sucrose, Glc H-5/C-5	3.84/75.1 (B)	–	–	–	3.86/75.2	3.84/75.1
Sucrose, Glc H-6/C-6	3.81/62.8 (B)	–	–	–	3.82/63.0	3.83/63.0
Sucrose, Fru H-1′/C-1′	3.67/64.0 (B)	–	–	–	3.69/64.3	3.70/64.3

Constituent and group	Reference δH/δC (source)	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Sucrose, Fru H-3′/C-3′	4.21/79.0 (B)	–	–	–	4.20/79.5	4.22/79.5
Sucrose, Fru H-4′/C-4′	4.04/76.7 (B)	–	–	–	4.05/76.9	4.06/76.9
Sucrose, Fru H-5′/C-5′	3.88/84.1 (B)	–	–	–	3.89/84.0	3.90/84.1
Sucrose, Fru H-6′/C-6′	3.81/65.1 (B)	–	–	–	3.83/65.1	3.83/65.1
Glucose (free), α-anomer, H-1/C-1 (with α reducing ends)	5.22/94.8 (B)	–	–	–	5.24/94.8	5.23/94.7
Glucose (free), β-anomer, H-1/C-1	4.63/98.6 (B)	–	–	–	–	4.66/98.6
Glucose (free), β-anomer, H-2/C-2	3.23/76.8 (B)	–	–	–	3.26/76.9	3.27/76.9
Glucose (free), β-anomer, H-3,H-5/C-3,C-5	3.47/78.5 (B)	–	–	–	3.49/78.6	3.51/78.3
Fructose (free), β-pyranose, H-6a/C-6	4.02/66.1 (B)	4.05/66.6	–	–	4.04/66.2	4.00/66.7
Fructose (free), β-pyranose, H-5/C-5	3.98/71.9 (B)	4.01/71.8	–	–	4.01/71.9	4.00/71.9
Fructose (free), α-furanose, H-5/C-5	4.06/84.1 (B)	–	–	–	4.06/84.1	4.06/84.3
Fructose (free), β-pyranose, H-4/C-4	3.88/72.4 (B)	–	–	–	3.89/72.7	–
Fructose (free), β-furanose, H-3/C-3	4.10/78.0 (B)	–	–	–	4.11/78.4	–
α-(1→4)-Glucan, internal residues, H-1/C-1	5.38/102.5 (B)	5.37/102.5	5.35/102.6	5.38/102.5	5.37/102.5	5.36/102.4
α-(1→4)-Glucan, internal residues, H-3/C-3	3.95/76.0 (B)	3.98/75.9	3.97/75.9	3.98/75.7	–	–
α-(1→4)-Glucan, internal residues, H-4/C-4 (4-O-substituted)	3.64/79.6 (B)	3.64/80.1	3.65/80.2	–	3.67/80.0	3.64/80.2
α-(1→4)-Glucan, internal residues, H-2/C-2	3.60/74.3 (B)	3.63/74.1	3.64/74.2	–	–	–
α-Glucan, non-reducing terminal residues, H-3/C-3	3.69/75.5 (B)	3.75/75.5	3.71/75.6	3.73/75.6	–	–
α-(1→4)-Glucan, internal residues, H-5/C-5	3.81/73.9 (B)	3.84/74.1	3.83/74.4	–	–	–
Hexose CH_2OH groups (α-glucan, trehalose, mannan and free sugars; unresolved), H-6/C-6	3.83, 3.76/63.2 (B)	3.84, 3.79/63.3	3.91, 3.85/63.4	–	3.87/63.4	–
α-(1→6) branch residue, H-1/C-1	4.97/101.3 (L)	4.97/101.1	4.98/101.1	4.96/101.0	4.97/101.0	4.97/100.9
Mannan (mannoprotein), terminal α-Man H-1/C-1	5.06/104.8 (L)	5.06/104.6	5.08/104.7	–	5.06/104.7	–
Mannan (mannoprotein), α-(1→3)-linked terminal Man H-1/C-1	5.15/104.8 (L)	–	5.16/104.7	–	5.15/104.9	–
Mannan (mannoprotein), →2)-α-Man H-1/C-1	5.27/103.2 (L)	5.25/102.9	–	5.25/103.2	–	–
Mannan (mannoprotein), →2,6)-α-Man H-1/C-1	5.12/100.9 (L)	5.15/100.6	5.10/101.0	–	5.12/100.7	–
Mannan (mannoprotein), 2-O-substituted Man H-2/C-2	4.06/80.8 (L)	3.98/80.5	4.14/80.9	–	–	–
Mannan (mannoprotein), Man H-2/C-2 (unsubstituted)	4.09/72.8 (L)	4.09/72.8	4.11/72.8	4.10/72.5	4.09/72.8	–
β-Linked glucosyl residue, H-1/C-1	4.49/105.4 (B)	–	4.54/105.4	–	–	–
β-Linked glucosyl residue, H-2/C-2	3.31/75.8 (B)	–	3.36, 3.33/75.7	–	–	–
β-Linked glucosyl residue, H-3/C-3	3.50/78.3 (B)	–	3.55, 3.50/78.4	–	–	–
Ethanol, CH_3	1.18/19.5 (B)	–	–	–	1.18/19.8	–
Ethanol, CH_2	3.65/60.1 (B)	–	–	–	3.66/59.9	–
2,3-Butanediol (tentative), CH_3	1.14/20.7	–	1.14/20.7	–	–	–
Nucleosides and nucleotides						
Uridine, H-6/C-6	7.88/144.6 (B)	–	7.85/144.6	–	–	–
Uridine, H-5/C-5	5.89/105.0 (B)	–	5.90/105.1	–	–	–

Constituent and group	Reference δH/δC (source)	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Uridine, H-1′/C-1′	5.91/92.0 (B)	5.90/92.0	5.90/91.8	5.92/91.8	5.93/92.0	–
Uridine, H-4′/C-4′	4.13/86.9 (B)	4.13/86.8	4.14/87.0	–	–	–
Uridine, H-2′/C-2′	4.34/76.4 (B)	–	4.35/76.2	–	–	–
Uridine, H-3′/C-3′	4.22/72.1 (B)	–	4.23/72.2	–	–	–
Adenosine-type purine nucleoside, H-1′/C-1′	6.06/91.1 (B)	6.07/90.9	6.04/91.0	–	–	–
Adenosine-type purine nucleoside, H-4′/C-4′	4.28/88.4 (B)	–	4.28/88.2	–	–	–
Adenosine-type purine nucleoside, H-2′/C-2′	4.77/76.6 (B)	4.78/76.4	4.75/76.5	–	–	–
Adenosine-type purine nucleoside, H-3′/C-3′	4.43/73.2 (B)	4.43/73.2	4.42/73.2	–	–	–
Guanosine-type nucleoside or nucleotide, H-1′/C-1′	5.90/90.0 (B)	5.91/90.3	5.88/90.4	–	–	–
5′-Nucleotide ribose (tentative), H-4′/C-4′	4.37/87.4 (B)	4.39/87.4	4.39/87.4	–	–	–
Lipid						
Unsaturated fatty acyl chain, CH=CH	5.32/131.9	–	–	5.31/132.0	–	–
Unsaturated fatty acyl chain, allylic CH_2	2.02/29.6	–	–	2.02/29.8	–	–
Unsaturated fatty acyl chain, $(CH_2)_n$	1.30/31.9	–	–	1.32/31.9	–	–

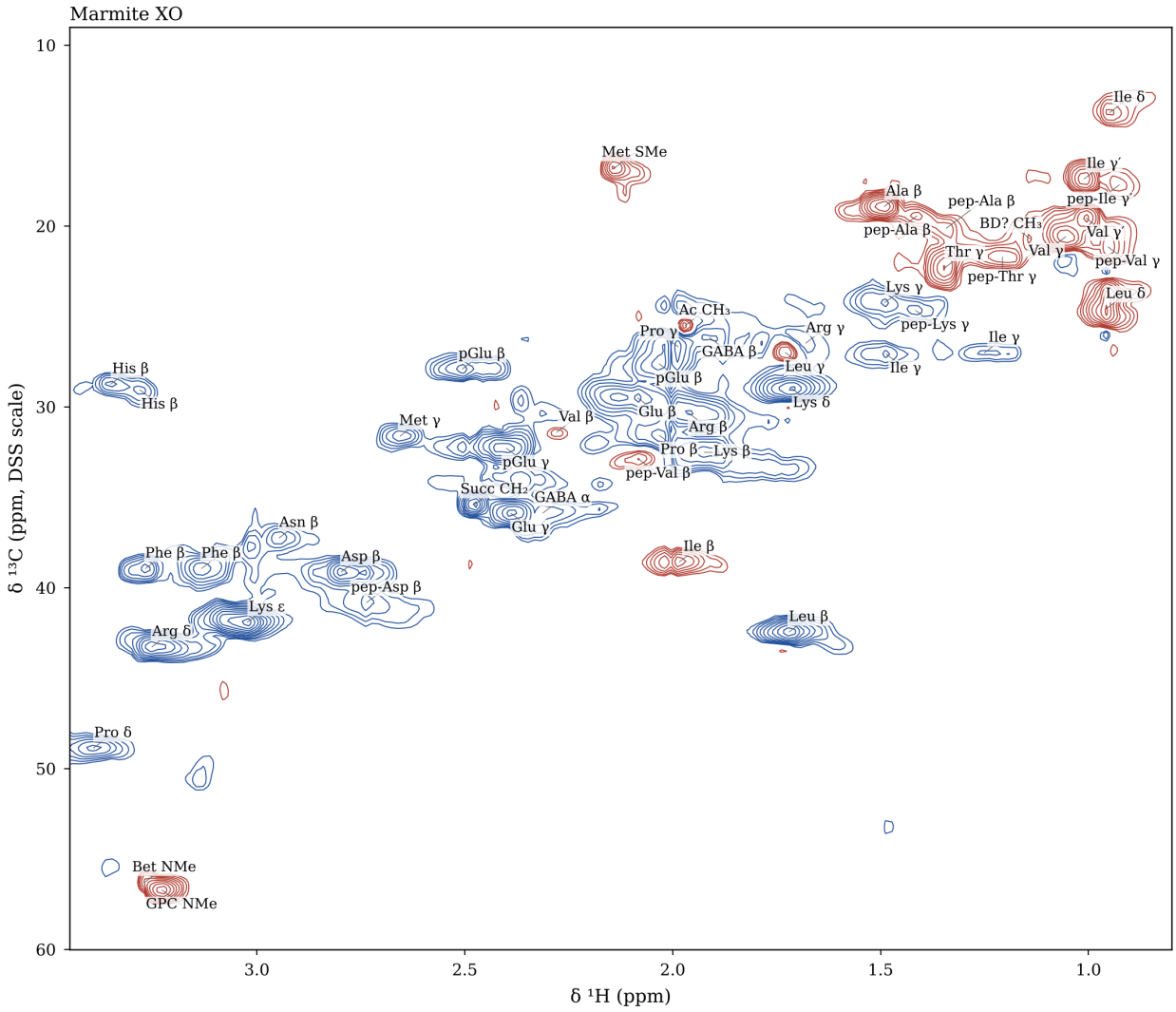


Figure 22. Aliphatic region of the multiplicity-edited gHSQC spectrum of Marmite XO, re-plotted from the numerical data with the assignments of Table 9. CH and CH_3 correlations are drawn in red and CH_2 correlations in blue; the ^{13}C axis is on the DSS scale. Abbreviations: pGlu, pyroglutamate; GPC, glycerophosphocholine; Bet, betaine; Succ, succinate; Ac, acetate; BD?, 2,3-butanediol (tentative); pep-, residue bound in a peptide.

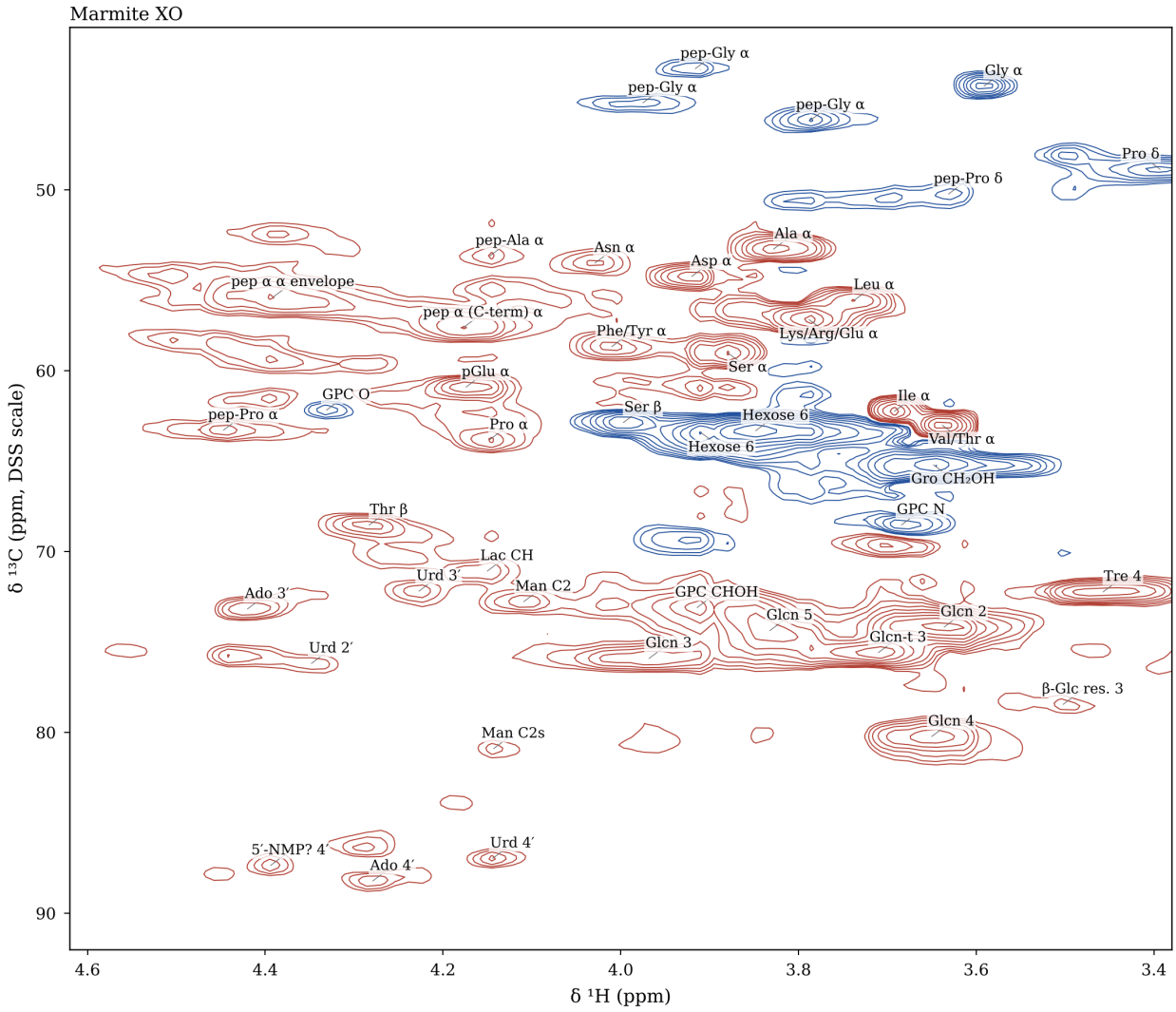


Figure 23. α-CH and carbinol region of the gHSQC spectrum of Marmite XO with assignments. Gro, glycerol; Hexose 6, the unresolved hydroxymethyl groups of the glucan, trehalose and other hexoses; GPC, glycerophosphocholine; Glcn, α-(1→4)-glucan (Glcn-t, non-reducing terminal residue); Tre, trehalose; Man, mannan; β-Glc res., β-linked glucosyl residue (tentative); Urd, uridine; Ado, adenosine-type nucleoside; Lac, lactate.

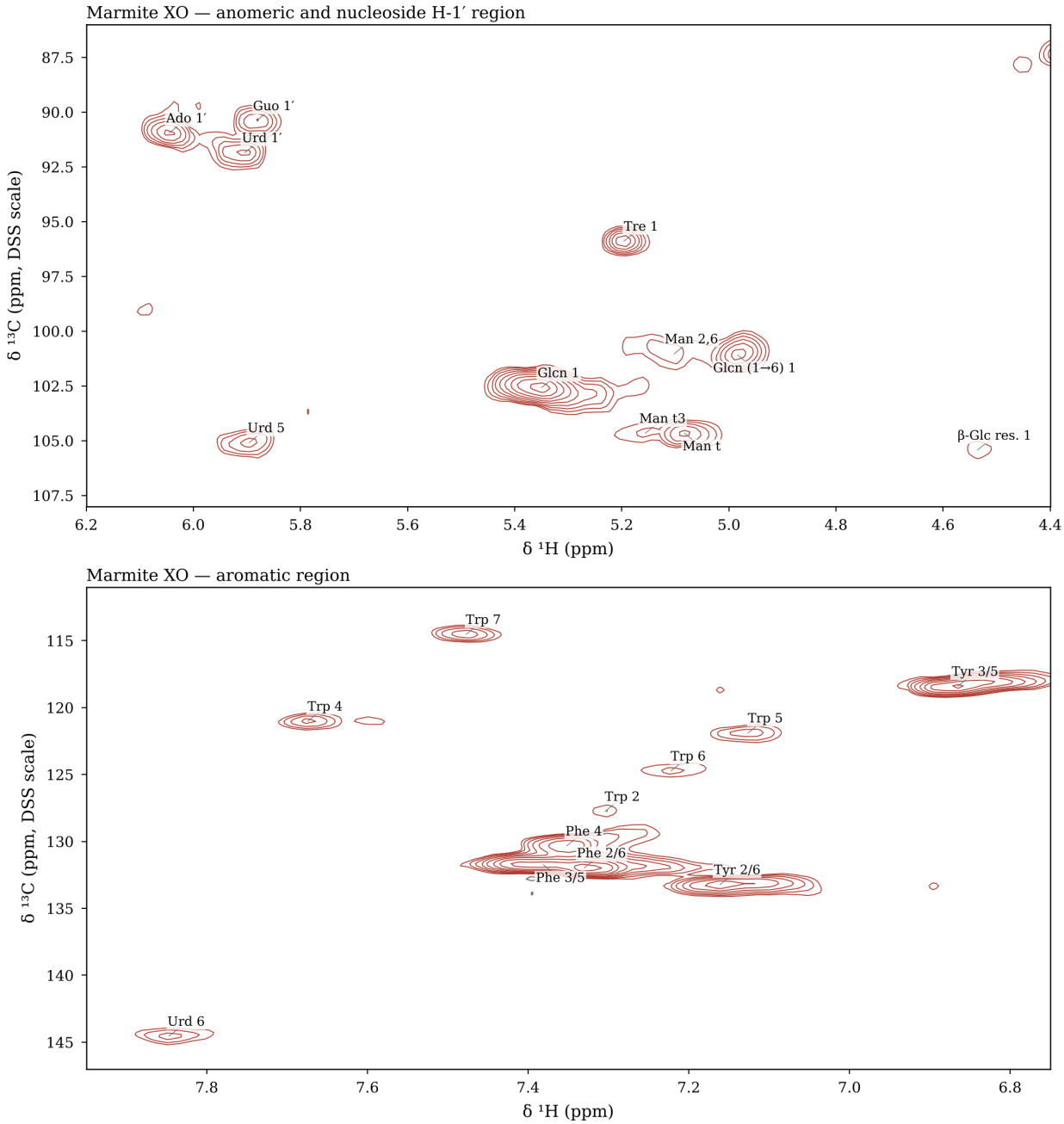


Figure 24. Anomeric and nucleoside H-1' region (top) and aromatic region (bottom) of the gHSQC spectrum of Marmite XO with assignments. The purine C-8 and C-2 and the histidine C-ε1 correlations are suppressed by the multiplicity editing and are absent.

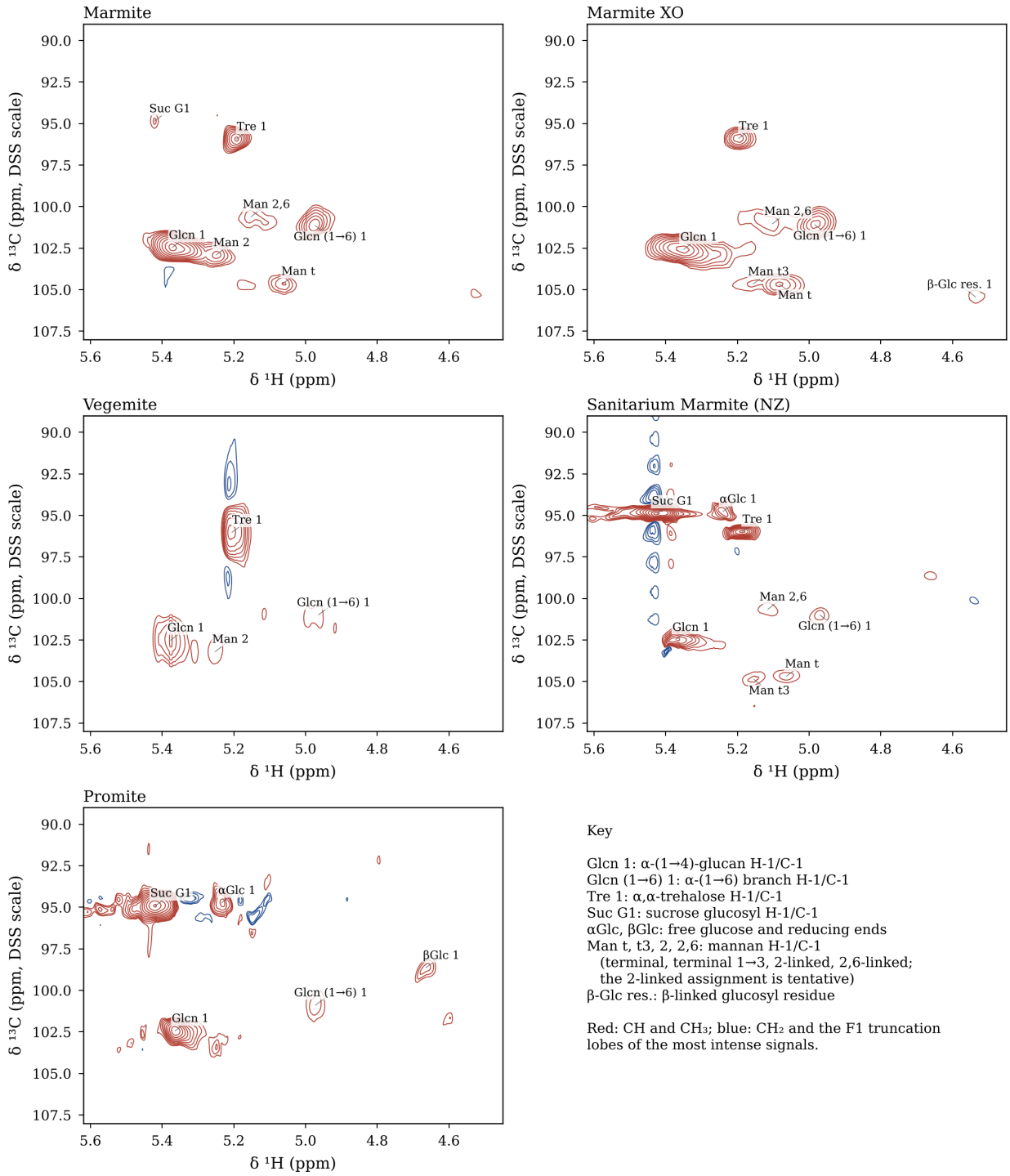


Figure 25. The anomeric region of the gHSQC spectra of the five products on common axes. The α-(1→4)-glucan and its α-(1→6) branch signal are common to all five; trehalose is present in the four yeast products and absent from Promite; sucrose and the reducing sugars mark the New Zealand Marmite and Promite; and the mannan anomeric signals are clearest in the two British products.

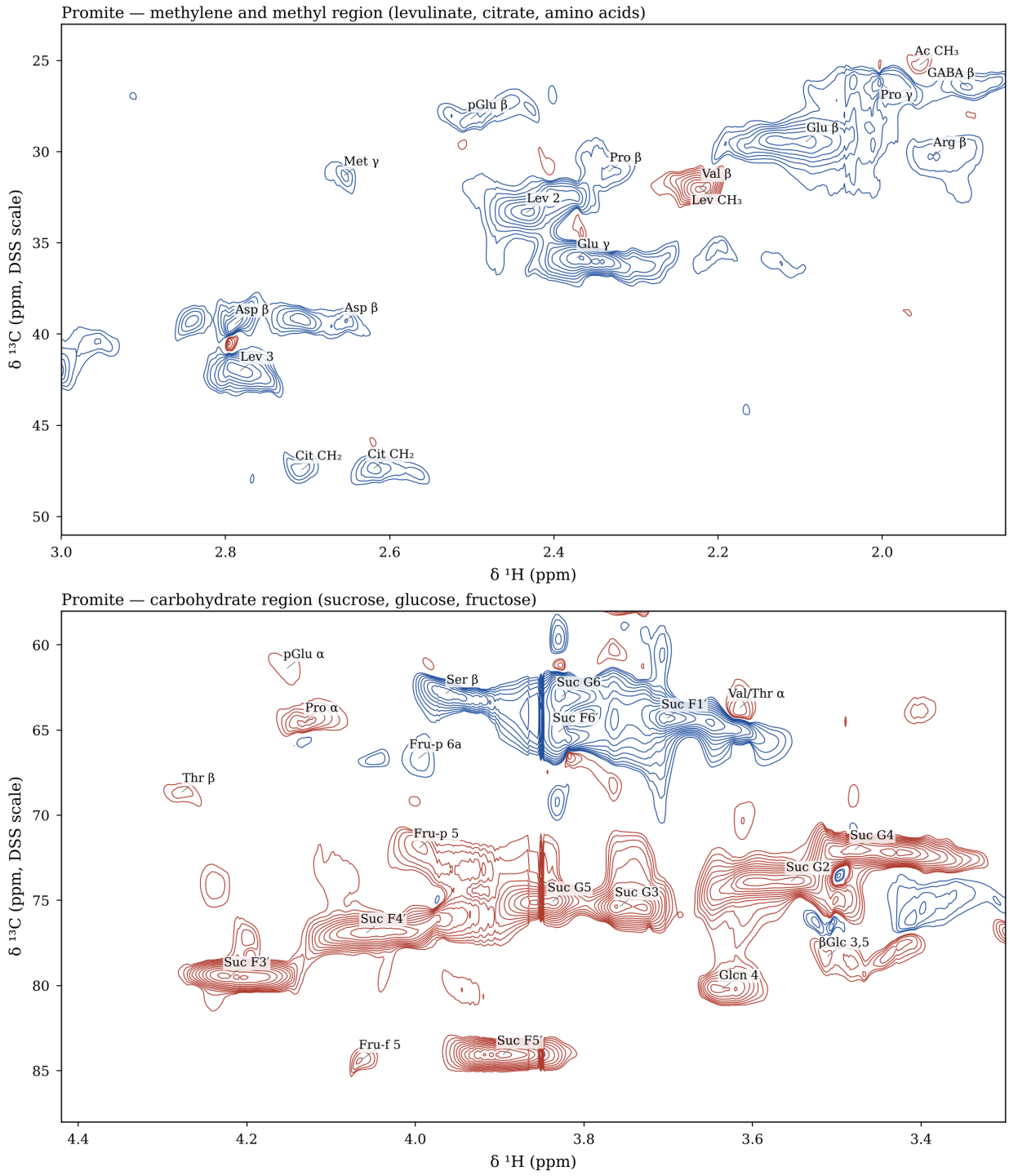


Figure 26. Regions of the gHSQC spectrum of Promite: the methylene and methyl region with the three correlations of levulinate (Lev) and the methylene pair of citrate (Cit), and the carbohydrate region with the assignments of sucrose (Suc; G, glucosyl; F, fructosyl), glucose and fructose.

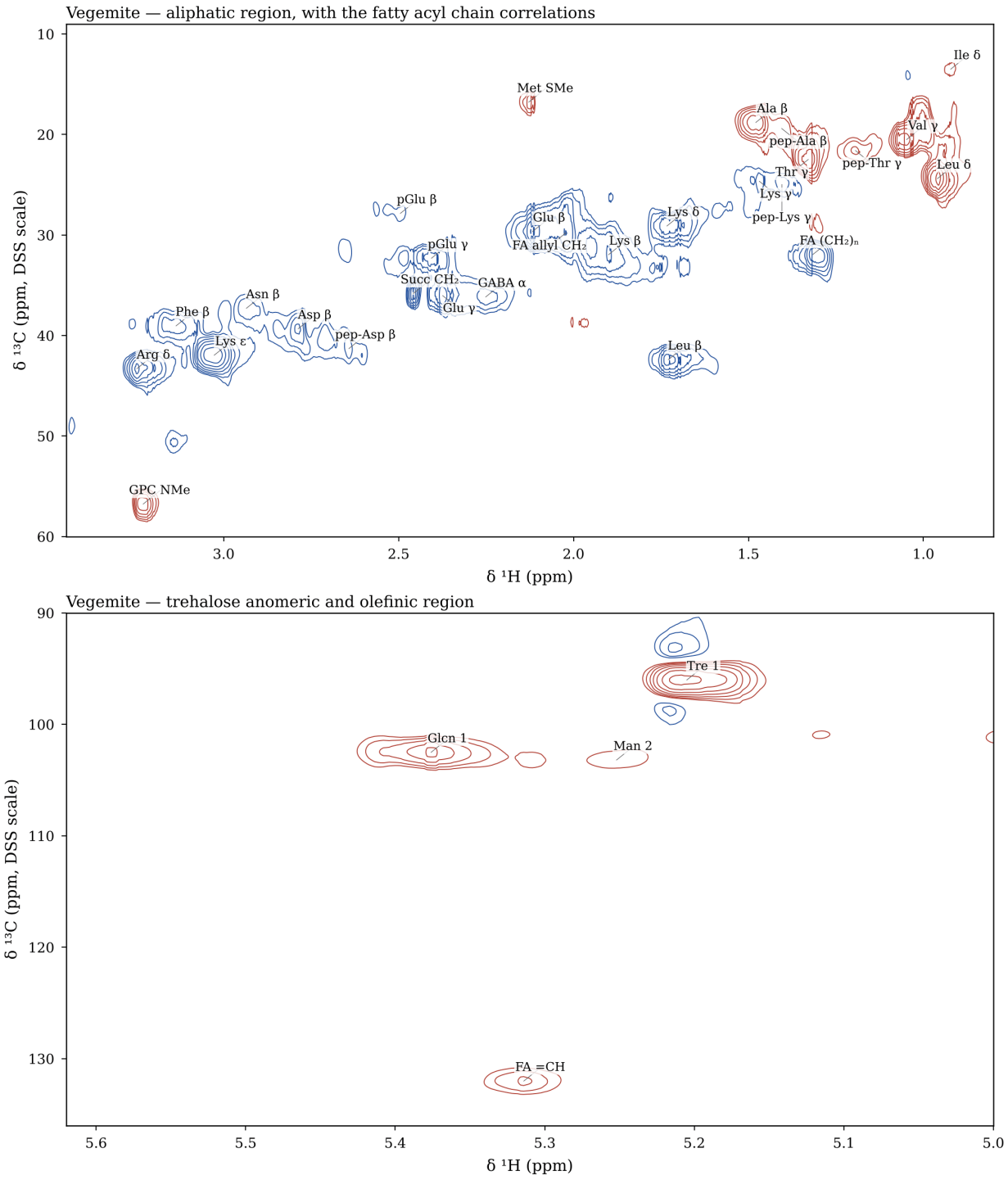


Figure 27. Regions of the gHSQC spectrum of Vegemite: the aliphatic region, in which the allylic methylene and chain methylene correlations of an unsaturated fatty acyl chain (FA) are marked, and the anomeric and olefinic region with the trehalose H-1/C-1 and the olefinic CH correlations. The blue responses above and below the trehalose signal are F1 truncation lobes.

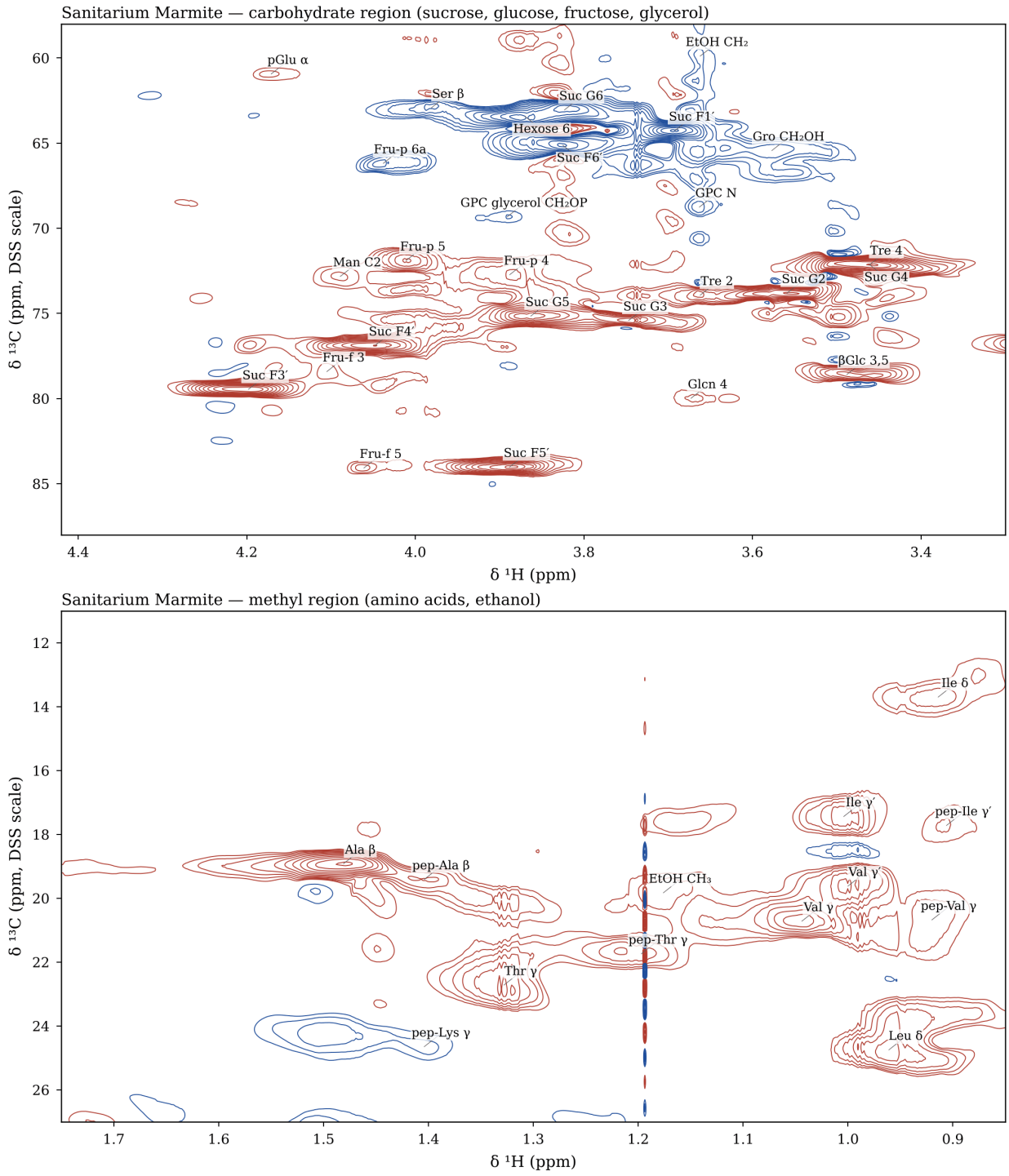


Figure 28. Regions of the gHSQC spectrum of the New Zealand (Sanitarium) Marmite: the carbohydrate region and the methyl region. The narrow vertical feature at δH 1.19 in the methyl region is the t_1 ridge of the ethanol methyl triplet.

8.12 Assignment of the gHMBC Spectra

The gHMBC spectra supply what the gHSQC cannot: the quaternary carbons, the connection of each proton to the carbons two and three bonds away, and with them the means of deciding between constituents whose one-bond correlations coincide. The 83 long-range correlations that were assigned are listed in Table 10

and the principal ones are drawn in Figures 29 and 30. In Marmite XO, whose spectrum is the most sensitive of the three, 67 of them are observed; in Vegemite, acquired with only 64 increments, 30; and in the New Zealand Marmite, where the sucrose correlations dominate, 33.

The carboxyl region is the most immediately useful. The alanine methyl protons correlate to the α-carbon at 53.3 ppm and to the carboxylate at 178.5 ppm in all three spectra, with the reverse correlation from the α-proton to the methyl carbon; the glycine methylene correlates to its carboxylate at 175.2 ppm; the aspartate β-protons to the side-chain carboxylate at 180.3 ppm and the asparagine β-protons to the amide carbon at 176.8 ppm, which separates the two; and the glutamate γ-protons to the side-chain carboxylate at 183.9 to 184.2 ppm. Acetate (1.94 to 1.97 ppm to 183.2 to 183.5 ppm) is confirmed in all three products, including Vegemite, in which its one-bond cross-peak had fallen below threshold, and succinate by correlations from its methylene protons both to the carboxylate (183.6 to 183.9 ppm) and to the symmetry-related methylene carbon at 35.3 to 35.7 ppm, the second of which is diagnostic of a symmetrical –CH_2–CH_2– unit. The 1.33 ppm doublet is shown to be composite in every product: in Marmite XO its dominant correlations are to carbons at 68.6 and 63.0 ppm, which are the β- and α-carbons of threonine, with a weaker one to a carboxylate at 184.1 ppm that belongs to lactate; in Vegemite the lactate correlations (185.5 and 71.0 ppm) are the stronger. A separate doublet at 1.21 ppm correlates to a carbon at 70.0 to 70.3 ppm, the β-carbon of a threonyl residue in a peptide, and a weak doublet at 1.58 ppm in Marmite XO to carbons at 51.8 and 173.3 ppm, the α-carbon and the amide carbonyl of an N-terminal alanyl residue; the peptide assignments of Section 8.11 are thereby confirmed independently.

Several assignments that could only be proposed from the one-bond data are settled. Methionine is secured by the three-bond correlation across sulfur in both directions, from the S-methyl protons at 2.13 ppm to the γ-carbon at 31.7 ppm and from the γ-protons at 2.65 ppm to the S-methyl carbon at 16.8 ppm. 4-Aminobutyrate is secured by correlations from the α-methylene protons at 2.33 ppm to carbons at 42.1 and 26.2 ppm, which are its γ- and β-carbons and which no glutamyl residue could supply. Pyroglutamate shows the correlations from its β-protons to the α-carbon at 60.8 ppm and from its γ-protons to the β-carbon at 28.1 ppm. Arginine is marked by the correlation of its δ-protons to the guanidino carbon at 159.2 ppm; phenylalanine by those of its β- and α-protons to the ring carbons at 132.0 and 137.7 ppm; tyrosine by that of H-2/6 to the phenolic carbon at 157.5 ppm. Glycerol is confirmed by the mutual correlations of its methylene and methine groups (3.58 ppm to 74.8 ppm; 3.64 and 3.80 ppm to 65.4 ppm), and the N-methyl protons of glycerophosphocholine correlate to the other N-methyl carbons at 56.7 ppm and to the N-methylene at 68.2 to 68.6 ppm in all three products, with the separate, weaker correlations of the choline N-methyl protons to 70.2 ppm and of the betaine N-methyl protons to 56.3 and 68.8 ppm in Marmite XO.

The most consequential single result concerns the sharp singlet at 8.60 to 8.64 ppm, which version 1.1 paired with the singlet at 8.22 ppm as the H-8 and H-2 of an adenine ring. In the gHMBC of Marmite XO the proton at 8.64 ppm correlates to carbons at 120.2 and 130.6 ppm. Those are the C-δ2 and C-γ of the imidazolium ring of histidine, reached from H-ε1; the H-8 of an adenine ring would correlate to C-4 and C-5 near 151 and 121 ppm, and no response is found at 151 ppm from this proton. The assignment is supported by the correlation of the histidine β-protons at 3.36 ppm to the same C-γ at 131.1 ppm and by lines at 136.86, 131.17, 120.24 and 28.63 ppm in the ^{13}C spectrum. The 8.6 ppm singlet of the British products is therefore the H-ε1 of free histidine in its protonated form, and it should not be counted as a nucleotide signal. The purine rings are nonetheless present, at lower intensity: a proton at 8.33 ppm correlates to a carbon at 150.9 ppm and one at 8.17 ppm to a carbon at 158.0 ppm, which are the H-8 to C-4 and H-2 to C-6 correlations of an adenine ring (the hypoxanthine ring of inosine would place C-6 at 161 ppm), and a proton at 8.00 ppm correlates to a carbon at 153.7 ppm, as does H-8 of a guanine ring. Uridine is confirmed by the correlations of H-6 to C-2 and C-4 (154.3 and 168.7 ppm) and of the H-1′ and H-5 protons to C-6 (144.5 ppm).

In the carbohydrate region the inter-residue correlations identify the disaccharides without recourse to shift tables. The anomeric proton of trehalose correlates to an anomeric carbon — 95.6 ppm in Marmite XO, 94.6 ppm in the coarser Vegemite spectrum — which, in a molecule with a single anomeric proton signal,

can only be C-1′ of the second, symmetry-related residue reached across the glycosidic oxygen; the same proton correlates to C-3 and C-5 at 75.0 to 75.3 ppm in all three products, the New Zealand Marmite included. In the New Zealand product the glucosyl H-1 of sucrose correlates across the glycosidic oxygen to the quaternary C-2′ of the fructosyl residue at 106.4 ppm, and the fructosyl H-1′ singlet, H-3′ and H-4′ give the full set of intra-residue correlations (to C-2′, C-3′, C-4′, C-5′ and C-6′). The free sugars are confirmed in the same spectrum by the correlation of the fructopyranose H-1 protons to the anomeric carbon at 100.7 ppm and of the β-glucose H-2 to C-1 at 97.8 ppm. A methyl doublet at 1.12 to 1.14 ppm correlates to a carbon at 74.1 to 74.4 ppm in Marmite XO and in the New Zealand Marmite, the basis of the tentative 2,3-butanediol assignment.

Table 10. Long-range ^{1}H–^{13}C correlations assigned in the gHMBC spectra of Marmite XO, Vegemite and the New Zealand Marmite (δH → δC in ppm; ^{13}C on the DSS scale). The figure in parentheses is the intensity of the response as a percentage of the most intense response of the same spectrum; –, not observed or not applicable.

Constituent	Correlation, proton (δH) → carbon (reference δC)	Marmite XO: δH → δC (rel. int.)	Vegemite	New Zealand Marmite
Alanine	H-β (1.48) → C-α (53.2)	1.50 → 53.3 (100)	1.49 → 53.1 (102)	1.48 → 53.2 (25)
Alanine	H-β (1.48) → COO^- (178.6)	1.49 → 178.5 (94)	1.48 → 178.7 (83)	1.48 → 178.6 (23)
Alanine	H-α (3.81) → C-β (18.8)	3.83 → 19.0 (11)	3.80 → 19.4 (19)	–
Valine	H-γ (1.04) → C-α (63.1)	1.05 → 62.9 (35)	–	0.99 → 63.0 (6)
Valine	H-γ (1.04) → C-β (31.8)	1.05 → 32.0 (22)	1.04 → 32.2 (45)	1.04 → 31.8 (5)
Valine	H-γ (1.04) → C-γ′ (19.4)	1.06 → 19.6 (25)	1.04 → 19.4 (41)	1.03 → 19.3 (8)
Valine	H-γ′ (0.99) → C-γ (20.7)	0.99 → 20.8 (29)	0.99 → 19.9 (33)	0.99 → 20.8 (7)
Valine	H-α (3.63) → C-β (31.8)	3.65 → 31.7 (7)	–	–
Leucine	H-δ (0.96) → C-β (42.5)	0.96 → 42.5 (98)	0.96 → 42.5 (83)	0.95 → 42.5 (11)
Leucine	H-δ (0.96) → C-γ (26.9)	0.96 → 26.7 (59)	0.96 → 27.5 (65)	0.95 → 27.0 (7)
Leucine	H-α (3.74) → C-β (42.5)	3.74 → 42.5 (8)	3.74 → 42.4 (5)	–
Leucine	H-α (3.74) → COO^- (178.4)	3.74 → 178.2 (7)	–	–
Isoleucine	H-γ′ (1.01) → C-β (38.6)	1.02 → 38.5 (24)	1.01 → 37.6 (7)	1.00 → 39.2 (2)
Isoleucine	H-γ′ (1.01) → C-γ (27.2)	–	1.01 → 28.2 (17)	–
Isoleucine	H-α (3.69) → COO^- (177.0)	3.69 → 176.9 (7)	–	–
Threonine	H-γ (1.33) → C-β (68.7)	1.35 → 68.6 (46)	1.33 → 67.4 (5)	1.33 → 68.4 (3)
Threonine	H-γ (1.33) → C-α (63.2)	1.34 → 63.0 (12)	1.32 → 64.5 (9)	–
Threonyl residue (peptide)	H-γ (1.21) → C-β (70.0)	1.21 → 70.3 (3)	1.20 → 70.0 (9)	–
Lactate	H-3 (1.34) → C-1 (COO^-) (185.3)	1.38 → 184.1 (5)	1.32 → 185.5 (17)	–
Lactate	H-3 (1.33) → C-2 (71.2)	–	1.32 → 71.0 (4)	–
Acetate	CH_3 (1.95) → COO^- (184.2)	1.97 → 183.2 (17)	1.94 → 183.5 (15)	1.95 → 183.4 (15)
Succinate	CH_2 (2.47) → COO^- (185.2)	2.47 → 183.6 (17)	–	2.45 → 183.9 (4)
Succinate	CH_2 (2.47) → CH_2 (symmetry partner) (36.9)	2.48 → 35.4 (13)	2.45 → 35.3 (6)	2.46 → 35.7 (7)
Glutamate	H-γ (2.37) → C-δ (COO^-) (184.1)	2.38 → 183.9 (18)	2.37 → 184.2 (36)	2.36 → 183.9 (15)
Glutamate	H-γ (2.37) → C-α (57.4)	2.38 → 58.0 (1)	2.36 → 57.0 (9)	2.36 → 57.5 (3)
Glutamate	H-γ (2.36) → C-β (29.7)	–	2.35 → 29.6 (12)	2.36 → 30.4 (2)
Pyroglutamate	H-β (2.52) → C-α (60.9)	2.50 → 60.8 (1)	–	–
Pyroglutamate	H-γ (2.42) → C-β (27.9)	2.42 → 28.1 (4)	–	–
4-Aminobutyrate (GABA)	H-α (2.33) → C-γ (42.0)	2.33 → 42.1 (7)	–	–
4-Aminobutyrate (GABA)	H-α (2.33) → C-β (26.4)	2.33 → 26.2 (3)	–	–
Methionine	S-CH_3 (2.13) → C-γ (31.5)	2.13 → 31.7 (7)	–	–
Methionine	H-γ (2.65) → S-CH_3 (16.6)	2.65 → 16.8 (6)	–	–
Methionine	H-γ (2.65) → C-α (56.6)	2.66 → 56.6 (3)	–	–
Aspartate	H-β (2.77) → C-γ (COO^-) (180.3)	2.77 → 180.3 (13)	2.73 → 180.8 (7)	–
Aspartate	H-β (2.78) → C-α (54.9)	2.77 → 54.6 (7)	–	–

Constituent	Correlation, proton (δH) → carbon (reference δC)	Marmite XO: δH → δC (rel. int.)	Vegemite	New Zealand Marmite
Asparagine	H-β (2.94) → C-γ ($CONH_2$) (177.2)	2.94 → 176.8 (7)	–	–
Asparagine	H-β (2.95) → C-α (54.0)	2.95 → 53.9 (6)	–	–
Lysine	H-ε (3.03) → C-δ (29.1)	3.03 → 29.1 (12)	3.02 → 30.4 (6)	–
Arginine	H-δ (3.23) → C-ζ (guanidino) (159.5)	3.23 → 159.2 (6)	–	–
Glycine	H-α (3.58) → COO^- (175.2)	3.59 → 175.2 (58)	3.57 → 175.0 (38)	3.58 → 175.5 (9)
Serine	H-α (3.88) → C-β (62.9)	3.88 → 63.0 (5)	–	–
Phenylalanine	H-β (3.12) → C-2/6 (132.1)	3.12 → 132.0 (6)	3.16 → 132.3 (8)	–
Phenylalanine	H-β (3.13) → C-1 (137.8)	3.13 → 137.7 (2)	–	–
Phenylalanine	H-2/6 (7.31) → C-β (39.1)	7.31 → 39.3 (3)	–	–
Phenylalanine	H-α (4.01) → C-1 (137.8)	4.01 → 137.7 (4)	–	–
Tyrosine	H-2/6 (7.16) → C-4 (157.7)	7.16 → 157.5 (7)	–	–
Tyrosine	H-3/5 (6.85) → C-1 (129.5)	6.85 → 129.2 (1)	–	–
Histidine (imidazolium)	H-ε1 (8.64) → C-δ2 (120.1)	8.64 → 120.2 (3)	–	–
Histidine (imidazolium)	H-ε1 (8.64) → C-γ (131.0)	8.64 → 130.6 (4)	–	–
Histidine (imidazolium)	H-β (3.36) → C-γ (131.0)	3.36 → 131.1 (1)	–	–
Tryptophan	H-2 (7.29) → C-3 (110.2)	7.29 → 109.6 (0)	–	–
Glycerophosphocholine	$N(CH_3)_3$ (3.23) → N-CH_3 (56.7)	3.24 → 56.7 (76)	3.22 → 56.6 (37)	3.22 → 56.6 (31)
Glycerophosphocholine	$N(CH_3)_3$ (3.23) → N-CH_2 (68.7)	3.24 → 68.6 (36)	3.22 → 68.4 (15)	3.22 → 68.2 (6)
Choline (minor)	$N(CH_3)_3$ (3.20) → N-CH_2 (70.1)	3.21 → 70.2 (4)	–	–
Betaine	$N(CH_3)_3$ (3.26) → N-CH_3 (56.0)	3.27 → 56.3 (6)	–	–
Betaine	$N(CH_3)_3$ (3.26) → CH_2 (68.8)	3.27 → 68.8 (9)	–	–
Glycerol	H-1 (3.57) → C-2 (74.8)	3.58 → 74.8 (14)	–	–
Glycerol	H-1 (3.64) → C-3 (65.2)	3.64 → 65.4 (8)	–	–
Glycerol	H-2 (3.80) → C-1/3 (65.2)	3.80 → 65.4 (5)	–	–
α,α-Trehalose	H-1 (5.21) → C-3/C-5 (75.0)	5.22 → 75.0 (2)	5.21 → 75.3 (45)	5.21 → 75.2 (9)
α,α-Trehalose	H-1 (5.21) → C-1′ (across the glycosidic oxygen) (95.9)	5.21 → 95.6 (1)	5.20 → 94.6 (12)	–
α,α-Trehalose	H-4 (3.45) → C-6 (63.2)	–	3.45 → 62.8 (10)	–
Sucrose	Glc H-1 (5.41) → Fru C-2′ (across the glycosidic oxygen) (106.4)	–	–	5.43 → 106.4 (21)
Sucrose	Glc H-1 (5.41) → Glc C-3/C-5 (75.2)	–	–	5.43 → 75.2 (34)
Sucrose	Fru H-1′ (3.68) → Fru C-2′ (106.4)	–	–	3.68 → 106.3 (103)
Sucrose	Fru H-1′ (3.68) → Fru C-3′ (79.0)	–	–	3.68 → 79.3 (69)
Sucrose	Fru H-3′ (4.22) → Fru C-4′ (76.7)	–	–	4.22 → 76.7 (88)
Sucrose	Fru H-4′ (4.05) → Fru C-3′ (79.0)	–	–	4.05 → 79.0 (34)
Sucrose	Fru H-4′ (4.05) → Fru C-6′ (65.1)	–	–	4.05 → 65.1 (28)
Sucrose	Fru H-4′ (4.05) → Fru C-5′ (84.1)	–	–	4.05 → 84.7 (10)
Sucrose	Glc H-4 (3.46) → Glc C-3/C-5 (75.2)	–	–	3.46 → 75.1 (29)
Sucrose	Glc H-4 (3.47) → Glc C-6 (62.8)	–	–	3.47 → 63.1 (12)
Fructose (free), β-pyranose	H-1 (3.56) → C-2 (100.8)	–	–	3.58 → 100.7 (5)
Glucose (free), β-anomer	H-2 (3.25) → C-1 (98.6)	–	–	3.25 → 97.8 (4)
Uridine	H-6 (7.86) → C-2 (154.5)	7.86 → 154.3 (5)	–	–
Uridine	H-6 (7.86) → C-4 (169.1)	7.86 → 168.7 (2)	–	–
Uridine	H-1′ / H-5 (5.89) → C-6 (144.6)	5.89 → 144.5 (1)	–	–
Adenine ring (adenosine-type)	H-8 (8.33) → C-4 (151.2)	8.33 → 150.9 (1)	–	–
Adenine ring (adenosine-type)	H-2 (8.17) → C-6 (158.4)	8.17 → 158.0 (1)	–	–
Guanine ring (guanosine-type)	H-8 (8.00) → C-4 (154.0)	8.00 → 153.7 (1)	–	–
2,3-Butanediol (tentative)	CH_3 (1.13) → CHOH (74.2)	1.14 → 74.1 (4)	–	1.12 → 74.4 (3)

Constituent	Correlation, proton (δH) → carbon (reference δC)	Marmite XO: δH → δC (rel. int.)	Vegemite	New Zealand Marmite
N-terminal alanyl residue (peptide)	H-β (1.58) → C-α (51.8)	1.58 → 51.8 (1)	–	–
N-terminal alanyl residue (peptide)	H-β (1.59) → C′ (amide) (173.4)	1.59 → 173.3 (1)	–	–

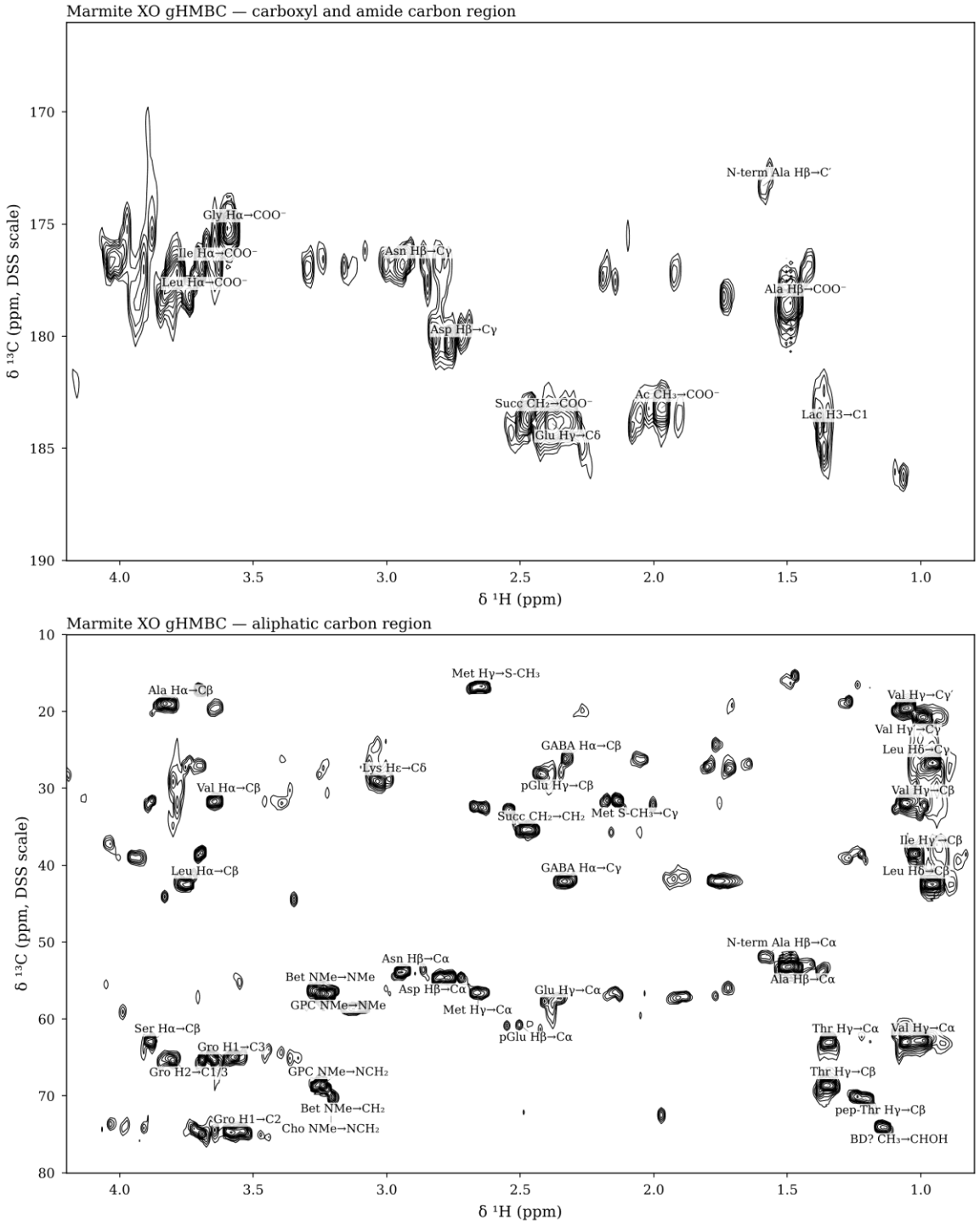


Figure 29. Regions of the gHMBC spectrum of Marmite XO, re-plotted from the numerical data (magnitude mode; ¹³C axis on the DSS scale): the carboxyl and amide carbon region and the aliphatic carbon region, with the assigned long-range correlations of Table 10. The responses at δH 3.21/δC 70.2 and at δH 3.27/δC 68.8, on either side of the glycerophosphocholine N-methyl to N-methylene correlation, are those of free choline (Cho) and of betaine (Bet).

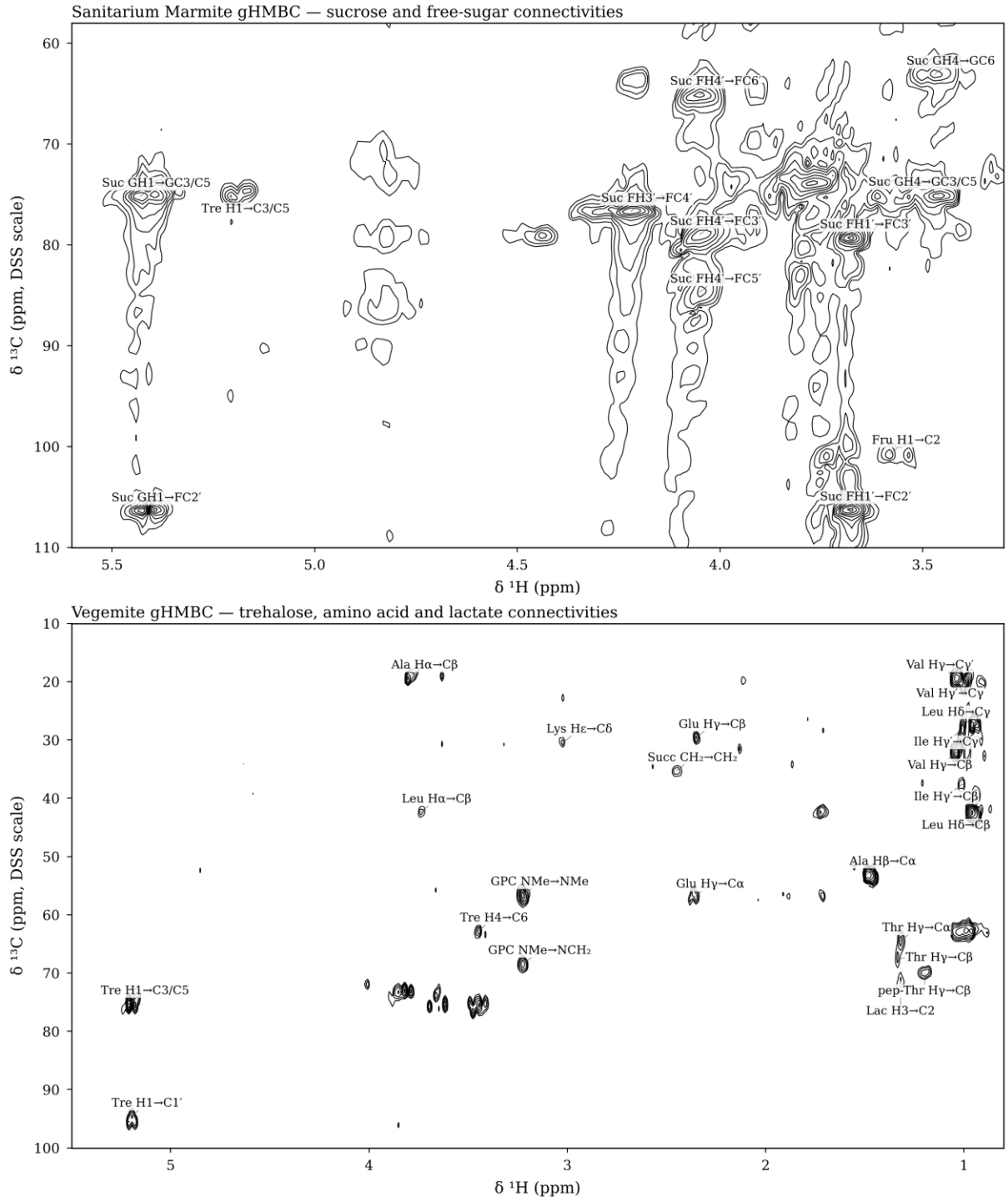


Figure 30. gHMBC spectra of the New Zealand Marmite (top; sucrose and free-sugar connectivities, including the inter-residue correlation from the glucosyl H-1 to the fructosyl C-2') and of Vegemite (bottom; trehalose, amino acid and lactate connectivities).

8.13 Assignment of the 2D J-Resolved Spectrum of Marmite XO

The J-resolved spectrum had been tilted and symmetrized in processing, so that each chemically distinct proton appears as a column of responses along F1 at its chemical shift, and its skyline projection onto F2 is

in effect a proton-decoupled ^{1}H spectrum. The F2 axis was aligned to the referenced one-dimensional spectrum (+0.017 ppm), and the F1 digital resolution is 1.7 Hz per point over a window of ±110 Hz, which limits the precision of the couplings read from it to about ±1.5 Hz. The multiplets that are resolved cleanly are listed in Table 11 and marked in Figure 31.

The singlets identify themselves on the zero-frequency row: formate at 8.46 ppm, the purine H-8 and H-2 protons at 8.34 and 8.21 ppm, glycine at 3.58 ppm, the N-methyl groups of betaine at 3.28 ppm, of glycerophosphocholine at 3.24 ppm and of choline at 3.21 to 3.22 ppm, succinate at 2.48 ppm, the methionine S-methyl at 2.14 ppm and acetate at 1.96 ppm. The presence of three N-methyl singlets, with zero-frequency responses in the ratio of roughly 9 : 1.6 : 1 for glycerophosphocholine, choline and betaine in this experiment, is the J-resolved counterpart of the ^{1}H, gHSQC and gHMBC evidence for one major and two minor trimethylammonium compounds. The doublets of the methyl region are separated according to their couplings — alanine at 1.50 ppm, the threonine and lactate methyls at 1.35 ppm, the valine pair at 1.06 and 1.00 ppm and the isoleucine γ′-methyl at 1.02 ppm, all with splittings of 7 to 7.6 Hz — and a weak doublet at 1.58 ppm is the methyl of the N-terminal alanyl residue found in the gHMBC. Two weak doublets at 1.15 and 1.11 ppm with splittings near 5.5 Hz are consistent with the methyl groups of the diastereomers of 2,3-butanediol and are entered as tentative. Triplets of 7.5 to 7.7 Hz mark the lysine ε-methylene at 3.04 ppm, the methionine γ-methylene at 2.66 ppm and, at 2.33 ppm, the α-methylene of 4-aminobutyrate, resolved from the glutamate and pyroglutamate γ-multiplets at 2.38 and 2.41 ppm. The methylene protons of glycerol give the expected pair of doublets of doublets, of which the upfield member at 3.56 ppm is resolved with couplings of 11.6 and 6.9 Hz, in agreement with the tabulated values;[90] the tyrosine doublets (8.7 Hz), the uridine H-6 and H-5 doublets (8.3 Hz) and the trehalose anomeric doublet (about 4 Hz) complete the list. The aspartate and asparagine β-methylene groups, which are strongly coupled at 300 MHz, give the expected displaced responses and were not analyzed.[70]

Table 11. Multiplets resolved in the homonuclear 2D J-resolved spectrum of Marmite XO (300 MHz, D_2O; tilted and symmetrized). Couplings were read from the F1 dimension (1.7 Hz per point) and are good to about ±1.5 Hz.

δH (ppm)	Multiplicity	J observed (Hz)	Assignment	Pattern expected of the free compound
8.46	s	—	Formate	singlet
8.34	s	—	Adenine-ring H-8 (adenosine-type nucleoside)	singlet
8.21	s	—	Adenine-ring H-2	singlet
7.89	d	8.3	Uridine H-6	d, ^{3}J(H-5,H-6) ≈ 8.1
7.42	t-like	7.4	Phenylalanine H-3/5	multiplet
7.32	d-like	6.5	Phenylalanine H-2/6	multiplet
7.18	d	8.7	Tyrosine H-2/6	d, ≈ 8.5
6.88	d	8.7	Tyrosine H-3/5	d, ≈ 8.5
5.91	d	8.3	Uridine H-5 (with H-1′)	d, ≈ 8.1 (H-1′ d, ≈ 4.5)
5.21	d	4.3	α,α-Trehalose H-1	d, ≈ 3.8
3.98, 4.00	ABX	≈ 11–12 (geminal), ≈ 5	Serine β-CH_2	two dd, J(gem) ≈ 12
3.66	dd (outer splitting resolved)	12.1	Glycerol H-1a	dd, 11.7 and 4.4
3.56	dd	11.6, 6.9	Glycerol H-1b	dd, 11.7 and 6.5
3.58	s	—	Glycine α-CH_2	singlet
3.28	s	—	Betaine $N(CH_3)_3$ (on the arginine δ and histidine β multiplets)	singlet
3.24	s	—	Glycerophosphocholine $N(CH_3)_3$	singlet
3.21	s	—	Choline $N(CH_3)_3$ (minor)	singlet
3.04	t	7.7	Lysine ε-CH_2	t, ≈ 7.6

δH (ppm)	Multiplicity	J observed (Hz)	Assignment	Pattern expected of the free compound
2.66	t	7.6	Methionine γ-CH_2	t, ≈ 7.6
2.48	s	—	Succinate CH_2	singlet
2.41	m (triplet-like)	7.7	Pyroglutamate γ-CH_2	multiplet
2.38	m (triplet-like)	7.7	Glutamate γ-CH_2	multiplet
2.33	t	7.5	4-Aminobutyrate α-CH_2	t, ≈ 7.4
2.14	s	—	Methionine S-CH_3 (on the glutamate β-CH_2 multiplet)	singlet
1.96	s	—	Acetate CH_3	singlet
1.58	d	7.6	N-terminal alanyl residue β-CH_3	d, ≈ 7.2
1.50	d	7.6	Alanine β-CH_3	d, 7.2
1.35	d	6.9	Threonine γ-CH_3 and lactate CH_3 (superposed)	d, 6.4 and 6.9
1.15; 1.11	d; d	5.5; 5.5	2,3-Butanediol CH_3, two diastereomers (tentative)	d, ≈ 6.2
1.06	d	7.3	Valine γ-CH_3	d, 7.0
1.02	d	7.1	Isoleucine γ′-CH_3	d, 7.0
1.00	d	7.6	Valine γ′-CH_3	d, 7.0
0.95–0.97	d and t (superposed)	7.5–7.9	Leucine δ-CH_3 (two d) and isoleucine δ-CH_3 (t)	d, 6.2; t, 7.4

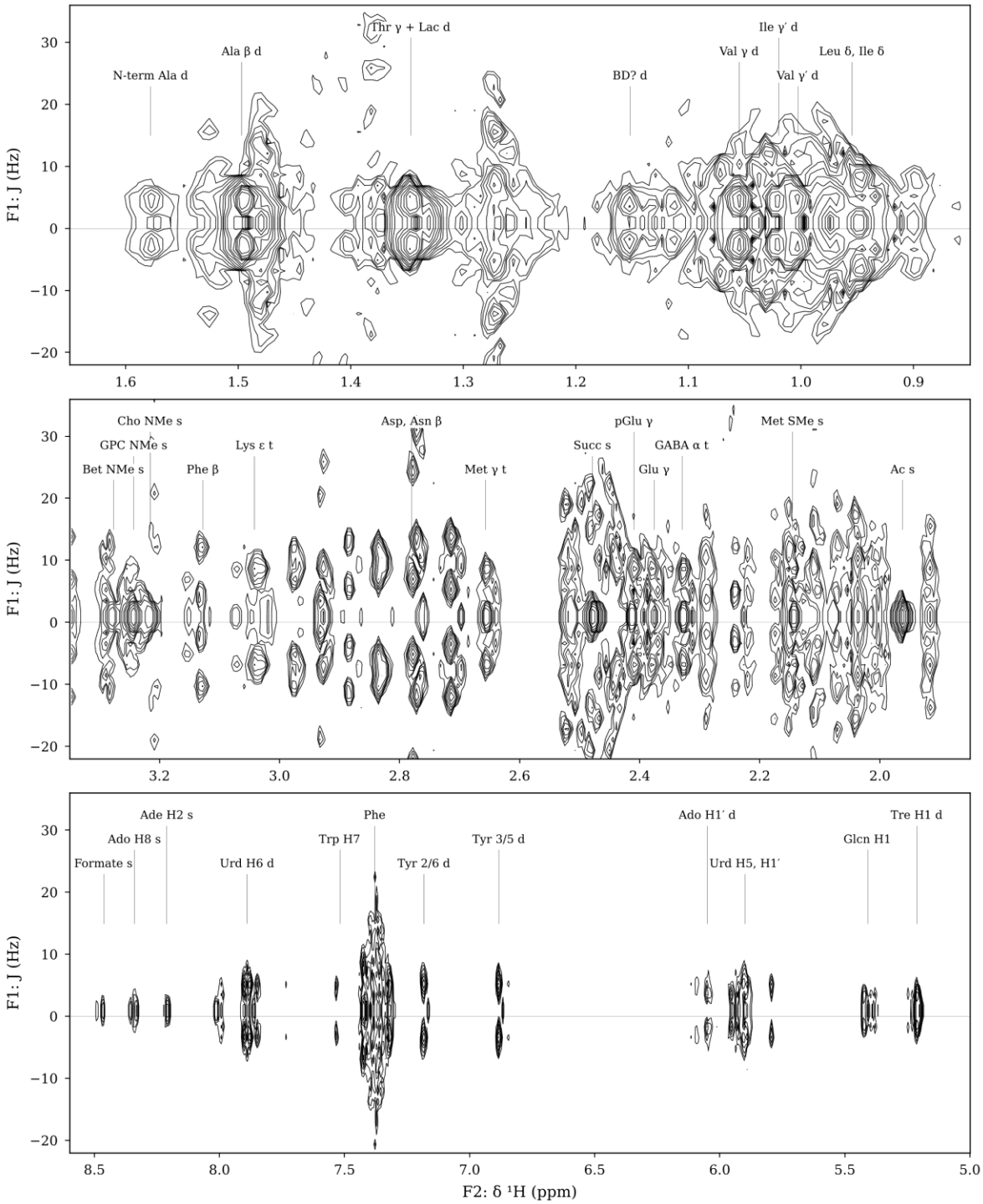


Figure 31. Regions of the homonuclear 2D J-resolved ¹H spectrum of Marmite XO (tilted and symmetrized; F1 in Hz), re-plotted from the numerical data with the multiplet assignments of Table 11.

8.14 The Chemistry Identified: Constituent Inventory by Product

Table 12 gathers the results of Sections 8.11 to 8.13 into an inventory in which each constituent is graded for each product. A constituent is entered as confirmed when it is supported by at least three one-bond correlations including one that is unique to it, or by one-bond correlations together with an independent

long-range, J-resolved or ^{13}C observation; as probable when two correlations, or one unique correlation with ^{13}C support, are found; and as tentative when it rests on a single correlation or on a coincidence of shift. Fifty constituents or structural classes are entered, one of them (the cytosine and uracil derivatives) only to record that it was sought and not found, and another (phosphocholine) because the data cannot decide it. The grading is necessarily more generous to Marmite XO, for which six experiments are available, than to Marmite, for which the gHSQC stands alone, and it should be read with that in mind.

The inventory recasts the comparison of the products in terms of constituents instead of spectral features. The four yeast products share a common core that is now defined in detail: sixteen to eighteen free amino acids, with pyroglutamate and 4-aminobutyrate beside them; a population of small peptides; glycerophosphocholine with a lesser amount of free choline (and, in the British products, betaine); glycerol; succinate, lactate and acetate; trehalose; a branched α-glucan; mannan; and uridine. Glycerophosphocholine is the deacylation product of the principal membrane phospholipid of the yeast, and both it and the choline to which it is hydrolyzed are well-characterized yeast metabolites;[92] their co-occurrence, in a ratio near three to one in all four products, is a signature of membrane lipid breakdown during autolysis that is common to the British and Australasian processes. Glycerol, the major by-product of yeast fermentation, arrives with the brewery yeast and is augmented by the same lipid hydrolysis.

Within that common core the British products are distinguished by betaine, by tryptophan, by free histidine, by the stronger mannan signals and by purine nucleosides in addition to uridine, and — taking the ratios of Section 8.11 — by an α-glucan content relative to alanine that in standard Marmite is some three times that of Vegemite or of the New Zealand product, or more. Marmite XO differs from standard Marmite in ways that are consistent with its longer digestion: between one-quarter and one-third of the trehalose and one-half of the glucan relative to alanine, and the fuller set of nucleoside correlations; the pyroglutamate-to-glutamate ratio, on the other hand, is the same in the two. Vegemite is distinguished by a trehalose content about four times that of Marmite on the same basis, by a small glucan background, by lactate as the stronger contributor to the 1.33 ppm signal and, uniquely, by the mobile monounsaturated fatty acyl chains. The New Zealand Marmite is the common core beneath sucrose, with trehalose at more than twice the proportion found in standard Marmite and a ratio of glucan to trehalose of the same order as that of Vegemite, with about one-eighth of the sucrose inverted to glucose and fructose, and with ethanol. Promite stands apart on every count: no cross-peak of trehalose, mannan, glycerophosphocholine, choline, succinate, uridine, asparagine or tryptophan is detected; aspartate, glutamate and proline are prominent and peptide-bound leucine is visible; and citrate, formate and the three-carbon pattern of levulinate are present. Each of those absences and presences follows from a formulation based on acid-hydrolyzed vegetable protein, sucrose and glucose syrup, and together they convert the conclusion of Section 8.7, which rested chiefly on the missing nucleotide signals, into one that rests on a dozen independent markers. The relative amounts on which these comparisons rest are collected in Table 13, each with the measurement from which it was taken.

Several of the identified constituents lend themselves to the process-diagnostic use proposed in Section 9.3 and to the authentication strategies of Section 10. The pyroglutamate-to-glutamate ratio records the thermal and storage history of the glutamine pool. The glycerophosphocholine-to-choline ratio and the nucleoside-to-nucleotide balance record the extent of phosphodiesterase and phosphatase action. The branch proportion and the terminal-residue content of the α-glucan offer a means of separating a native dextrin from an added maltodextrin or glucose syrup, and the ratio of glucan residues to trehalose separates the British from the Australasian yeast extracts by an order of magnitude; the ratio of glucose and fructose to sucrose measures inversion. The ratio of free to peptide-bound alanine is a degree-of-hydrolysis index read from three resolved methyl signals. And the set formed by asparagine, tryptophan, trehalose, glycerophosphocholine and uridine on the one hand, and levulinate and formate on the other, discriminates a yeast autolysate from an acid hydrolysate more robustly than any single marker. Betaine, confined here to the two British products, is a further discriminator, although one whose origin is not yet understood. All of these are at present qualitative or semi-quantitative observations on single lots; their conversion into specifications requires the quantitative, replicated and buffered data of the program in Section 11.

Table 12. Constituents identified in the five products from the gHSQC, gHMBC, 2D J-resolved, ^{1}H, ^{13}C and DEPT data, with the grade of each identification. C, confirmed; P, probable; T, tentative; –, not detected; ob, obscured by a coincident signal. The criteria for the grades are given in the text.

Constituent	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite	Principal evidence
Free amino acids						
Alanine	C	C	C	C	C	HSQC β, α; HMBC H-β→C-α, COO^{-}; 2D-J
Valine	C	C	C	C	C	HSQC γ, γ′, β; HMBC
Leucine	C	C	C	C	C	HSQC δ, γ, β, α; HMBC
Isoleucine	C	C	C	C	P	HSQC δ, γ′, γ, β; HMBC
Threonine	C	C	C	C	C	HSQC γ, β; HMBC H-γ→C-β, C-α
Serine	C	C	C	C	C	HSQC β, α; HMBC
Glycine	C	C	C	C	C	HSQC; HMBC to COO^{-}; 2D-J singlet
Proline	C	C	P	P	C	HSQC α, δ, β, γ; ^{13}C
Lysine	C	C	C	C	C	HSQC ε, δ, γ, β; HMBC; 2D-J
Arginine	C	C	C	C	P	HSQC δ, β, γ; HMBC H-δ→C-ζ
Glutamate	C	C	C	C	C	HSQC γ, β; HMBC H-γ→C-δ
Glutamine	–	T	–	–	–	^{13}C only (XO); γ-CH_2 not found
Aspartate	C	C	C	C	C	HSQC β, α; HMBC H-β→C-γ
Asparagine	C	C	C	C	–	HSQC β, α; HMBC H-β→$CONH_2$; absent from Promite
Methionine	C	C	P	C	P	HSQC S-CH_3, γ; HMBC across S
Phenylalanine	C	C	C	C	C	HSQC ring, β; HMBC to C-1
Tyrosine	C	C	C	C	C	HSQC ring; HMBC H-2/6→C-4
Tryptophan	P	C	–	–	–	HSQC ring CH (3 and 5 correlations)
Histidine (imidazolium form)	P	C	T	–	–	HSQC β; HMBC H-ε1→C-δ2, C-γ; ^{13}C
4-Aminobutyrate	T	C	T	T	T	HSQC β, α; HMBC H-α→C-γ, C-β; 2D-J triplet
Pyroglutamate	C	C	C	C	P	HSQC α, γ, β; HMBC; ^{13}C
Peptide-bound residues (Ala, Thr, Val, Ile, Leu, Lys, Pro, Gly, Asp)	C	C	P	P	P	HSQC at random-coil positions; HMBC (Thr, N-terminal Ala)
Trimethylammonium compounds						
Glycerophosphocholine	C	C	P	C	–	HSQC N-CH_3, N-CH_2, O-CH_2, CH_2OP; HMBC; $^{2}J(C,P)$ doublet
Choline (free, minor)	P	C	P	P	–	second N-CH_3 singlet (^{1}H, 2D-J); HMBC to 70.2 ppm
Betaine	P	C	–	–	–	third N-CH_3 singlet (^{1}H 3.27; 2D-J); HSQC N-CH_3 at 56.2–56.3; HMBC to 56.3 and 68.8 ppm; DEPT CH_3 line at 56.03 ppm
Phosphocholine	ob	ob	ob	ob	–	not assessable: N-CH_3 coincides with glycerophosphocholine, O-CH_2 (4.16/60.9) with pyroglutamate α-CH of opposite sign; ^{31}P NMR required
Organic acids						
Lactate	P	C	C	P	T	HSQC CH; ^{13}C; HMBC H-3→C-1, C-2
Acetate	T	C	C	C	P	HSQC CH_3; HMBC to COO^{-}; 2D-J singlet
Succinate	C	C	C	C	–	HSQC CH_2; HMBC to COO^{-} and to CH_2; 2D-J singlet

Constituent	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite	Principal evidence
Citrate	–	–	–	T	C	HSQC AB CH_2; ^{13}C C-3 at 78.5 ppm
Formate	P	P	P	P	C	^{1}H singlet; aliased HSQC response (Promite); 2D-J
Levulinate	–	–	–	–	P	three HSQC correlations; three ^{13}C lines; ketone carbon not detected
Carbohydrates and polyols						
Glycerol	C	C	P	P	ob	HSQC CH_2 pair, CH; ^{13}C 65.2, 74.7; HMBC; 2D-J
α,α-Trehalose	C	C	C	C	–	HSQC H-1/C-1 and ring; HMBC H-1→C-1′; ^{13}C
Sucrose	T	–	–	C	C	HSQC, all protonated carbons; HMBC H-1→C-2′; ^{13}C C-2′
Glucose (free; α and β)	–	–	–	C	C	HSQC anomeric and β ring; ^{13}C; HMBC
Fructose (free)	T	–	–	C	P	HSQC β-pyranose, α-furanose; ^{13}C C-2; HMBC
α-(1→4)-Glucan (branched dextrin)	C	C	P	C	C	HSQC H-1/C-1, C-3, linked C-4; ^{13}C
α-(1→6) branch residues	C	C	T	T	T	HSQC H-1/C-1 4.97/101.1
Reducing ends of maltooligosaccharides	–	–	–	P	T	^{13}C line at 98.46 ppm beside that of free β-glucose (New Zealand Marmite); α-anomeric ^{1}H doublet in excess of the ^{13}C estimate of free glucose
Mannan (mannoprotein)	C	C	P	P	–	HSQC anomeric and C-2 correlations; ^{13}C
β-Linked glucosyl residue (β-glucan)	–	T	–	–	–	three weak HSQC responses (4.54/105.4, 3.33–3.36/75.7, 3.50–3.55/78.3–78.5); no HMBC or ^{13}C anomeric support
Ethanol	–	–	–	C	–	HSQC CH_3, CH_2; ^{13}C
2,3-Butanediol	–	T	–	T	–	HSQC CH_3 (XO); HMBC from the methyl protons to 74 ppm; 2D-J doublets (XO); no reference spectrum compared
Nucleic acid constituents						
Uridine	P	C	T	T	–	HSQC base and ribose; HMBC H-6→C-2, C-4; 2D-J
Adenosine-type purine nucleoside	P	C	–	–	–	HSQC ribose; HMBC and ^{13}C of the adenine ring
Guanosine-type nucleoside or nucleotide	T	P	–	–	–	HSQC H-1′/C-1′; HMBC H-8→C-4
5′-Nucleotides	T	T	–	–	–	HSQC C-4′ only; 5′-CH_2 not established
Cytidine, cytosine, uracil	–	–	–	–	–	not observed
Lipid						
Monounsaturated fatty acyl chains	–	–	C	–	–	HSQC CH=CH, allylic CH_2, $(CH_2)_n$

Table 13. Relative amounts of selected constituents and the measurements on which they rest. Molar ratios from the ^{1}H spectra (90° pulse, recycle time 8.3 s; spectra as processed) are, for the sharp doublets, the mean of two treatments over limits set 4 Hz beyond the outer lines — two Lorentzian lines on a straight local baseline, and direct integration above a straight line between the limits — with the range of the six evaluations made at 3, 4 and 5 Hz given in parentheses where it is quoted; the N-methyl singlets were fitted as single Lorentzian lines on a straight local baseline, and the glucan envelopes were integrated above a straight line through flanking windows; ^{13}C entries are ratios of line heights of like carbons; gHSQC

entries are ratios of cross-peak volumes or heights of like signals. All values refer to single lots and are estimates (see Section 12). n.d., not detected; n.e., not evaluated because of overlap.

Quantity and basis	Marmite	Marmite XO	Vegemite	New Zealand Marmite	Promite
Trehalose per alanine, mol/mol (^{1}H; range of the six evaluations in parentheses)	0.29 (0.27–0.33)	0.08 (0.08–0.09)	1.25 (1.15–1.37)	0.68 (0.64–0.73)	n.d.
α-(1→4)-Glucan residues per alanine, mol/mol (^{1}H envelope)	2.3	1.2	≤ 0.8	≈ 0.8	n.e.
α-Glucan residues per trehalose molecule (^{1}H)	9	15	≤ 0.6	≈ 1.2	–
α-Glucan residues per trehalose molecule (gHSQC anomeric volumes)	12	19	1.7	1.9	–
Branch residues, fraction of the α-glucan anomeric total (gHSQC volumes ± noise s.d.)	0.200 ± 0.006	0.216 ± 0.008	0.23 ± 0.02	0.24 ± 0.05	0.14 ± 0.02
Branch residues, fraction of the α-glucan anomeric total (^{1}H envelopes)	0.13	0.12	n.e.	n.e.	n.e.
Glycerophosphocholine per alanine, mol/mol (^{1}H singlet fits)	0.12	0.12	0.08	0.18	n.d.
Free choline relative to glycerophosphocholine (^{1}H singlet areas)	0.43	0.29	0.30	0.25	–
Betaine relative to glycerophosphocholine (^{1}H singlet areas)	0.64	0.26	n.d. (< 0.2)	n.d. (< 0.2)	–
Sucrose per alanine, mol/mol (^{1}H; range in parentheses)	n.e. (trace)	n.d.	n.d.	6.5 (6.3–6.9)	7.9 (7.4–8.8)
Trehalose per sucrose, mol/mol (^{1}H; ^{13}C C-1 line heights)	–	–	–	0.10; 0.10	–
Free glucose per sucrose (^{13}C β-C-1 line height, corrected for the anomeric proportion)	–	–	–	0.13	0.06
Free fructose per sucrose (^{13}C β-pyranose C-2 line height, corrected for the tautomeric proportion)	–	–	–	0.15	0.04
α-Anomeric glucose H-1 (free glucose and reducing ends) per sucrose H-1 (^{1}H)	–	–	–	0.07	0.05
Pyroglutamate relative to glutamate (heights of the γ-CH_2 gHSQC cross-peaks)	1.0	1.1	0.6	0.5	–
Pyroglutamate relative to glutamate (^{13}C line heights of the α, β and γ carbons)	–	0.89–1.23	0.45–0.75	0.55–0.80	–
Check of the gHSQC: trehalose-to-alanine ratio from cross-peak volumes divided by that from the ^{1}H spectrum	2.2	3.0	1.2	1.0	–
Check of the gHSQC: N-methyl groups (all trimethylammonium compounds) relative to alanine, cross-peak volumes divided by ^{1}H areas	1.4	1.4	2.0	1.3	–
Check of the gHSQC: sucrose H-1 relative to alanine, cross-peak volumes divided by ^{1}H areas	–	–	–	0.57	0.69

8.15 Revisions to the Version 1.1 Reading, and Signals Remaining Unassigned

The numerical data confirm most of what was read from the figures, and the instances in which they do not are set out in Table 14 so that the two versions can be reconciled. The more important are the scale of the ^{13}C axes; the identity of the tallest line in the ^{13}C spectrum of Marmite XO, which is glycerol; the identity of the 8.6 ppm singlet, which is histidine; the speciation of the trimethylammonium signal, which includes betaine in the two British products; the form in which the RNA-derived material is present in the British products; and the trehalose content of the New Zealand Marmite, which is that of a trehalose-rich extract

of the Vegemite type and not a trace. The last two bear on the argument of Section 8.9 — the former weakening one of its inferences and the latter extending another — and a paragraph to that effect has been added there. A further revision concerns method and not chemistry: the cross-peak volumes of the gHSQC spectra were found, by comparison with the ^{1}H spectra, not to be proportional to amount between signals of unlike kind, and no statement of relative amount in this version rests on them alone.

Of the 591 cross-peaks retained in the five gHSQC spectra, 447 are accounted for by the entries of Table 9. Of the 144 that are not, 26 are secondary maxima lying within 0.07 ppm in ^{1}H and 1.2 ppm in ^{13}C of an assigned cross-peak of the same phase, and the remainder are listed, with their intensities, in the companion workbook. In the yeast products they include a methylene at 2.37/34.1 — the strongest of them in Marmite, and in the position expected of the γ-methylene of a glutaminyl residue bound in a peptide or of a γ-glutamyl group; methylene responses at 3.14/50.6 and 3.50/48.1; a methine at 3.70/69.7 that may belong to the mannan; a methylene at 3.79/66.7 that may belong to glycerol 3-phosphate; a methine at 4.44/75.8; and a group of weak α-CH responses between δH 4.3 and 4.5 that are evidently peptide residues not individually identified. In the New Zealand Marmite and in Promite they include a methine at 5.15/74.1, which is the strongest unassigned response of Promite, methylene responses at 2.96 to 2.99/40.4 and, in Promite, at 3.77/55.6. Around the most intense sucrose signals of those two spectra there are in addition decoupling sidebands, truncation lobes and t_1 ridges that pass the intensity threshold and that were recognized as artifacts by their position and shape. None of the unassigned responses exceeds about one-tenth of the most intense signal of its spectrum, but several are specific to one product and are for that reason of potential diagnostic interest; total correlation spectroscopy on buffered solutions, and spiking with the candidates named above, are the means of resolving them. Weak sharp lines at 8.63, 8.55 and 8.49 ppm in the ^{1}H spectra of Vegemite, the New Zealand Marmite and Promite, for which histidine H-ε1 and purine H-8 protons are candidates, give no detectable two-dimensional correlation and are likewise unassigned.

Table 14. Readings of version 1.1 that were revised in version 1.2 on the evidence of the numerical data.

Subject	Version 1.1 (read from the figures)	Version 1.2 (numerical data)
Scale of the ^{13}C axes	Marmite XO ^{13}C and all gHSQC F1 axes on the TMS scale (alanine CH_3 at 17.0 ppm); DEPT axes offset by about 2 ppm	Alanine CH_3 reads 15.75 ppm (Marmite XO ^{13}C) and 16.3–16.4 ppm (gHSQC); corrections to the DSS scale are +3.11 and +2.40 to +2.55 ppm; the DEPT axes lie within 0.25 ppm of the DSS scale; the gHMBC axes require +2.85 to +5.05 ppm (Table 8)
Tallest line of the Marmite XO ^{13}C spectrum	C-6 of the glucan and trehalose	Glycerol CH_2 (65.18 ppm); the C-6 carbons lie at 63.2 ppm
Singlet at 8.60–8.64 ppm	H-8 of an adenine ring (5′-AMP or adenosine)	H-ε1 of free histidine, imidazolium form (HMBC to 120.2 and 130.6 ppm)
Trimethylammonium singlet near 3.24 ppm	Choline and glycerophosphocholine, undifferentiated	Three singlets resolved: glycerophosphocholine (3.24 ppm, major), free choline (3.21 ppm, 0.25–0.4 of it in all four yeast products) and betaine (3.27 ppm; 0.6 of the glycerophosphocholine singlet in Marmite and 0.25 in Marmite XO; not detected in the Australasian products); phosphocholine cannot be assessed from these data
RNA-derived constituents of the British products	Nucleotides, nucleosides and bases; adenine, hypoxanthine and guanine rings; uridine and cytidine	Uridine, an adenosine-type and a guanosine-type nucleoside established; cytidine, cytosine and uracil not found; 5′-nucleotides tentative; purine ring CH invisible to the edited gHSQC
Trehalose in the products	Minor in Marmite, weaker in XO, dominant in Vegemite, trace in the New Zealand Marmite	Trehalose per alanine (mol/mol, ^{1}H) about 0.29, 0.08, 1.2 and 0.7 respectively: the New Zealand product is a trehalose-rich extract of the Vegemite type; one molecule of trehalose per ten of sucrose there
Doublet near 5.2 ppm, New Zealand Marmite	Trehalose or the α-reducing end of maltodextrin	Both resolved: trehalose 5.19/96.0 and α-glucose 5.24/94.8
Sucrose in the New Zealand Marmite and Promite	Present; inversion not assessed	Free glucose and fructose identified; about one-eighth of the sucrose inverted in the New Zealand product, less in Promite

Subject	Version 1.1 (read from the figures)	Version 1.2 (numerical data)
Soluble α-glucan	Glycogen and/or wort dextrin	Branched dextrin: branch residues 0.12–0.13 (^{1}H) to 0.20–0.24 (gHSQC) of the anomeric total in the yeast products, 0.14 (gHSQC) in Promite; limit dextrin probable, intact glycogen not excluded; glucan residues per trehalose about 9–19 in the British products and 2 or fewer in Vegemite and the New Zealand Marmite; mannan identified beside it
Maltodextrin in the New Zealand Marmite	Declared corn maltodextrin taken to be visible as the anomeric shoulder near 5.4 ppm and the branch-point cross-peak	The α-glucan has the branch proportion and the glucan-to-trehalose ratio of the yeast products to which none is added; an added maltodextrin is not distinguishable within it
Relative amounts	Visual estimates from the figures (Table 6)	Line fits and matched integrals of the ^{1}H spectra (recycle time 8.3 s) and ^{13}C line heights; gHSQC volumes shown to differ from the ^{1}H ratios by factors of up to three between unlike signals and used for like signals only
Pyroglutamate	Listed among the minor multiplets	Major constituent; γ-CH_2 cross-peak equal to that of glutamate in Marmite and Marmite XO
Olefinic response of Vegemite	Tentative; confirmation by allylic CH_2 and chain signals awaited	Confirmed: CH=CH 5.31/132.0, allylic CH_2 2.02/29.8, $(CH_2)_n$ 1.32/31.9; monounsaturated chains
Levulinate in Promite	Tentative (methyl singlet; acetone not excluded); ketone carbon said to lie outside the acquired window	Probable: CH_3, CH_2 and CH_2 correlations and three ^{13}C lines; acetone excluded as the source of the pattern; the spectrum extends to 271 ppm and the ketone carbon is not detected above noise
Formate in the gHSQC	Not discussed	Observed in Promite as an F1-aliased response (δC 174.1 after unfolding); nearly nulled by the editing
Succinate position in Vegemite	2.41 ppm in the ^{1}H spectrum of 2021 (near neutral)	2.46 ppm in the gHSQC of the 2023 dissolution; apparent pH of all two-dimensional samples near 5
Betaine as a marker of yeast source (Section 10.4)	A singlet pair at 3.27 and 3.90 ppm described as diagnostic of a molasses-grown yeast if present	The 3.27 ppm singlet is present in both British products; betaine is not a specific marker of yeast source without a reference population, the vegetable juice concentrate and the cereal wort being alternative sources
Branching of beer dextrins (Section 10.1)	Branch-to-linear anomeric ratio said to be far higher for glycogen than for maltodextrin or for beer dextrins	The limit dextrins of beer are heavily branched; the four yeast products show between one residue in eight and one in five at a branch point

9. NMR for Quality and Consistency Monitoring

9.1 A Quantitative NMR Analyte Panel for Yeast-Extract Spreads

The first application of NMR to a spread or an industrial extract is the replacement of several targeted assays by a single quantitative measurement. Table 15 proposes an analyte panel, with the signal to be integrated, its multiplicity, its principal interferences and its feasibility at 60 MHz, chosen from the assignments of Table 5 and from the experience of Section 8. The panel covers the umami and kokumi fraction (glutamate, glutamine, the nucleobase sum, and glutathione where resolvable), the process-indicator amino acids (alanine, glycine, the branched-chain aggregate, lysine, the aromatic pair), the microbial and thermal indicators (lactate, acetate, succinate, formate, ethanol), the yeast-reserve carbohydrates (trehalose, α-glucan), the formulation carbohydrates (sucrose, glucose, maltodextrin) and the phospholipid-derived choline compounds. All are referenced to an internal standard of known concentration and reported per gram of paste and, after a separate moisture determination, per gram of dry matter.[8,9]

Table 15. Proposed qNMR analyte panel for yeast-extract spreads (^{1}H, D_2O, phosphate buffer pH 7.0, TSP or DSS internal standard).

Analyte	Signal integrated (δ, mult., nH)	Field needed	Interferences / remarks	Quality attribute
Glutamate	2.34 m, γ-CH_2, 2H	300 MHz (HSQC preferred)	Glutamine 2.45, pyroglutamate, proline; deconvolution	Umami strength; deamidation

Analyte	Signal integrated (δ, mult., nH)	Field needed	Interferences / remarks	Quality attribute
Glutamine	2.45 m, γ-CH_2, 2H	300 MHz	Glutamate; succinate at low pH	Extent of deamidation (Gln/Glu)
Alanine	1.48 d, CH_3, 3H	60 MHz	Lysine/leucine CH_2 shoulder	Proteolysis marker; dominant free AA
Glycine	3.56 s, CH_2, 2H	300 MHz	Glycerol, sugar ring H	Proteolysis marker
Val + Leu + Ile (aggregate)	0.94–1.05, CH_3, ~15H per set	60 MHz (aggregate)	Ile γ-CH_2 1.25; individual AA need TOCSY	Hydrophobic AA; bitterness precursors
Lysine	3.02 t, ε-CH_2, 2H	300 MHz	GABA 3.01, Tyr β-CH_2	Maillard substrate (loss on heating)
Tyrosine; phenylalanine	6.90 d, 2H; 7.32–7.43 m, 5H	300 MHz	Tyramine (Tyr); Trp	Aromatic AA; tyramine screening
Nucleobase sum (H-8/H-2)	8.0–8.65, singlets, 1H each	300 MHz (60 MHz aggregate)	Formate 8.46; niacin 8.62/8.28	Nucleolysis; 5′-nucleotide route; I+G addition
Nucleoside H-1′	5.9–6.15 d, 1H each	300 MHz	Uracil H-5	Nucleotide speciation
Trehalose	5.19 d, H-1, 2H	60 MHz	Reducing-end α-glucose 5.23; maltose 5.23	Yeast physiology; autolysis (trehalase) end-point
α-(1→4)-Glucan (as glucose units)	5.35–5.42 br, H-1, 1H/unit	60 MHz	Sucrose 5.41; maltose non-reducing H-1′	Brewery dextrin / glycogen residue; maltodextrin addition
Sucrose	5.41 d, H-1, 1H (or 4.22 d, 1H)	60 MHz	α-Glucan envelope (use 4.22 d)	Sugar addition
Glucose	5.23 d + 4.65 d (α + β), 1H	300 MHz	Trehalose 5.19; reducing ends	Glucose syrup addition; glycogen/trehalose hydrolysis
Choline + GPC	3.20–3.23 s, $N(CH_3)_3$, 9H	60 MHz	Betaine 3.27; Arg δ-CH_2 3.24	Phospholipid turnover; yeast origin
Succinate	2.41 s (pH 7), CH_2, 4H	60 MHz	Glutamine at low pH	Yeast metabolism; process consistency
Acetate	1.92 s, CH_3, 3H	60 MHz	—	Thermal/microbial indicator
Lactate	1.33 d, CH_3, 3H (or 4.11 q)	300 MHz	Threonine 1.32 d (use HSQC or 4.11 q)	Bacterial contamination of spent yeast
Formate	8.46 s, CH, 1H	60 MHz	—	Thermal / acid-hydrolysis marker
Ethanol	1.18 t, CH_3, 3H	60 MHz	—	Residual beer; fermentation
Glutathione (GSH)	2.95 dd, Cys β-CH_2, 2H	300 MHz (HSQC)	Asp/Asn β-CH_2; Tyr β	Kokumi fraction; redox state
Levulinate	2.22 s, CH_3, 3H	300 MHz	Acetone 2.22; Met 2.13	Acid-HVP marker
Total 5′-nucleotide P; cyclic nucleotide P; phosphate; GPC; polyphosphate	^{31}P: 3–5 ppm; 20–21 ppm; ~2.5 ppm; ~0 ppm; ~−20 ppm	300 MHz ^{31}P	pH-dependent shifts; use buffer and EDTA	Nuclease route; I+G addition; phosphate speciation

9.2 Fingerprint-Based Statistical Process Control

The second application is the use of the whole spectrum as a multivariate fingerprint. The spectrum is divided into buckets of 0.02 to 0.04 ppm, the water and reference regions are excluded, the bucket integrals are normalized to the internal standard (or, for pure consistency monitoring, to total intensity), the resulting data matrix is mean-centered and Pareto-scaled, and principal component analysis is applied to a training set of accepted production lots.[93,94] Pareto scaling — division of each mean-centered variable by the square root of its standard deviation — is preferred to unit-variance scaling for spectral data because it retains the weighting of the major signals while allowing minor but consistent signals to contribute, and because it does not inflate noise regions; the choice of scaling has been shown to change the biological information content of a metabolomic data set materially, and it should be fixed in the method and not varied lot by lot.[93] New lots are projected onto the model and judged by Hotelling's T^2 (distance within the model plane) and by the Q-residual (distance from the model plane), with control limits set from the training set; a lot that exceeds either limit is flagged, and the contribution plot of its residual identifies the spectral region — and therefore the constituent — responsible. This is the scheme that has been operated for fruit juices on a commercial scale for more than a decade, and it transfers to yeast extracts without modification of principle.[10,62] The hydrolysate study of Combe and co-workers is the closest published precedent: it found that lot-to-lot differences were carried by a small subset of metabolites, which is precisely the situation in which a residual-based control chart is more sensitive than a battery of single-analyte specifications.[7]

Three practical points deserve emphasis. Buffering and consistent loading are prerequisites, because pH-driven shifts of succinate, glutamate, citrate and the nucleobases would otherwise dominate the first principal component. Spectral alignment (for example by correlation-optimized warping or by icoshift-type segment alignment) should be applied before bucketing when the buffer does not fully suppress shift variation. And the model must be built on lots that span the accepted process range, including seasonal variation of the spent yeast; a model built on a single month's production will flag normal seasonal variation as deviation. For a spread manufacturer, an additional and attractive use of the fingerprint is the monitoring of maturation: a series of spectra along the seven-day (or, for XO, 28-day) maturation would define a trajectory in principal-component space, and the end-point of maturation could be defined objectively as the point at which the trajectory reaches the accepted release region.

9.3 Process-Diagnostic Ratios

Between the single-analyte panel and the whole-spectrum fingerprint lies a third level of information: ratios between constituents that report on specific process steps. The ratio of free alanine (or of the branched-chain aggregate) to the peptide envelope, obtainable from DOSY-filtered or from CPMG-filtered spectra in which the small molecules and the peptides are separated by diffusion or by relaxation, is an NMR analog of the amino-nitrogen to total-nitrogen ratio and reports the degree of hydrolysis. The glutamine-to-glutamate ratio reports the extent of deamidation and therefore the thermal history and the glutaminase activity. The trehalose-to-α-glucan ratio and the absolute trehalose reports the physiological state of the yeast and the completeness of trehalase action, as the Marmite/Marmite XO comparison suggests. The ratio of nucleobases plus nucleosides to intact nucleotides (from ^{1}H H-1′ signals, or from ^{31}P) reports the phosphatase activity and distinguishes an endogenous-nuclease product from a phosphodiesterase product. The lactate and acetate levels report the hygiene of the spent yeast and the length of storage before processing, since lactic acid bacteria are the principal contaminants of brewery yeast.[30] GABA, from its 3.01/2.30/1.90 ppm triplets, reports glutamate decarboxylase activity during autolysis.[30] Each of these ratios is dimensionless, is independent of the moisture content and of the internal standard, and can be trended lot by lot; a set of five or six of them constitutes a compact process signature that would be difficult to obtain by any other single technique.

10. Adulteration Detection and Product Identity

10.1 Carbohydrate Dilution

The most economically obvious adulteration of a yeast extract or spread is extension with cheap carbohydrate — sucrose, glucose syrup, maltodextrin or malt extract — and it is also the easiest to detect, because each of these materials has an intense, isolated anomeric signal that is absent from an unadulterated autolysate. The New Zealand product and Promite in Section 8 are, in effect, worked examples: sucrose is identified unambiguously by its 5.41/4.22 ppm doublets and its 104 ppm quaternary carbon, maltodextrin by the 5.4 ppm envelope with its 100 ppm anomeric carbon and its 79 to 80 ppm linked C-4, and glucose syrup by the α/β anomeric pair. Where the addition is declared, the NMR measurement verifies the dose; where it is undeclared, the measurement is the evidence. The one ambiguity is between added maltodextrin and the native brewery dextrin or glycogen of a brewer's-yeast extract, both of which give the same α-(1→4)/α-(1→6) pattern. Three approaches resolve it: DOSY, which distinguishes the molecular-mass distribution of a corn maltodextrin (dextrose equivalent 10 to 20, mean chain length 5 to 10 glucose units) from that of glycogen (10^6 to 10^7 Da) and of beer dextrins (predominantly 4 to 15 units); the ratio of α-(1→6) branch anomeric signal to α-(1→4) signal, which is higher for glycogen (about 8 to 9 percent branching) and for the limit dextrins of beer than for a starch-derived maltodextrin (4 to 5 percent) — in the four yeast products examined here it lies between one residue in eight and one in five (Section 8.11), which corrects the statement of version 1.1 that beer dextrins are sparsely branched; and the quantitative level, since a brewer's-yeast autolysate rarely carries more than a few percent of dextrin whereas a formulation addition is typically 5 to 20 percent of the paste.[37,54,71] The New Zealand product is the test case. Its α-glucan has the branch proportion and the glucan-to-trehalose ratio of the yeast products to which no maltodextrin is added, so that on the second and third of these criteria the declared maltodextrin is not detected as such; the first, a measurement of molecular size, has not been made.

10.2 Substitution by Hydrolyzed Vegetable Protein

Acid-hydrolyzed vegetable protein (HVP) is the closest functional substitute for a yeast extract — it is rich in free glutamate and in the other free amino acids, it is dark, and it is cheaper — and its partial or complete substitution for yeast extract is the adulteration that a purchaser of "yeast extract" most needs to exclude. Three independent NMR lines of evidence address it, and Promite illustrates all three. First, HVP contains no nucleotides, nucleosides or purine bases, because plant storage protein is hydrolyzed without any accompanying RNA, whereas a yeast extract cannot be made without releasing 5 to 15 percent of its dry mass as RNA derivatives; the 8.0 to 8.65 ppm and 5.9 to 6.15 ppm regions, and the ^{31}P spectrum, are therefore the primary discriminators, and a "yeast extract" whose nucleobase-to-amino-acid ratio falls below the range of authentic products has been diluted with a non-yeast protein hydrolysate. Second, acid hydrolysis produces the carbohydrate-degradation markers levulinic acid and formic acid, which are absent from an enzymatic autolysate at comparable levels, and it destroys tryptophan, so that the tryptophan signals at 7.5 to 7.75 ppm are absent; levulinic acid has served for decades as the marker by which chemically hydrolyzed soy sauce is distinguished from brewed soy sauce, and its 2.22 ppm methyl singlet is one of the cleaner signals in a spread spectrum.[64,65] Third, the amino acid profile of an HVP differs from that of a yeast extract in characteristic ways — glutamine and asparagine are quantitatively converted to glutamate and aspartate, proline is prominent in wheat-gluten HVP, and the free/peptide ratio is close to unity in a fully hydrolyzed HVP — which are visible in the ratios of Section 9.3. Where HVP is suspected, the 3-MCPD analysis of Section 5.8 provides an orthogonal chromatographic confirmation, since the chloropropanols arise specifically from the hydrochloric acid hydrolysis of lipid-containing protein sources.[59]

10.3 Added Monosodium Glutamate and Added 5′-Ribonucleotides

Candor is required on two points. Solution NMR cannot distinguish glutamate that was liberated from yeast protein from glutamate that was added as monosodium glutamate: the molecule is the same, and its chemical

shifts are the same. What NMR can do is to place the glutamate level in the context of every other free amino acid. In an autolysate the glutamate is accompanied by alanine, leucine, lysine, valine and the other amino acids in proportions set by the yeast proteome and by the process, and the glutamate-to-alanine, glutamate-to-branched-chain and glutamate-to-total-free-amino-acid ratios of authentic products fall within a definable range; a product whose glutamate lies far outside that range while its other amino acids do not has, in all probability, been fortified. That inference is statistical, not chemical, and it requires a reference population. A definitive answer requires isotope-ratio methods — the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of fermentation-derived monosodium glutamate made from cane or corn sugar differ from those of glutamate derived from yeast grown on a C_3 substrate — and those lie outside the scope of solution NMR.

Added disodium 5′-inosinate and 5′-guanylate, by contrast, are more tractable, because the native nucleotide fraction of an autolysate is a mixture of 2′-, 3′- and 5′-esters, cyclic esters, nucleosides and bases of all four bases, whereas the commercial additive contributes only the 5′-esters of inosine and guanosine. A ^{31}P spectrum in which 5′-IMP and 5′-GMP are present at high level while the 2′,3′-cyclic and 3′-nucleotide signals are absent or trivial indicates either a product made by the phosphodiesterase route or a product to which I+G has been added, and the ^{1}H spectrum resolves the two cases by the presence, in the phosphodiesterase product, of the 5′-esters of adenosine, cytidine and uridine as well, which are not present in the additive.[34] A "natural" claim that rests on the absence of added I+G is therefore testable by NMR.

10.4 Substitution of Yeast Source

A spread that is marketed as a brewer's-yeast product may be made, in whole or in part, from primary-grown baker's-type yeast or from *Cyberlindnera* utilis, and a customer or a regulator may wish to know. The brewery origin leaves several marks. The dextrin envelope of Section 10.1, the barley proteins detected by proteomics, the cereal allergen declaration and the residual ethanol, maltose and maltotriose of an attenuated wort are all absent from a primary-grown yeast extract, whose carbohydrate residue is trehalose, glycogen fragments and mannan alone.[24,29] Conversely, a molasses-grown yeast can carry betaine, which is abundant in beet molasses and is taken up by the yeast, and which gives a characteristic pair of singlets at 3.27 and 3.90 ppm that would be diagnostic if present. The numerical analysis of version 1.2 found the first of these singlets, with its N-methyl carbon at 56.2 to 56.3 ppm and its long-range correlation to a methylene carbon at 68.8 ppm, in both British products and in neither Vegemite nor the New Zealand Marmite (Section 8.11). It cannot be read as evidence of a molasses-grown yeast without further work, because the vegetable juice concentrate declared for the British product and the betaine of the cereal wort carried in with a brewery yeast are alternative sources; what the observation does establish is that betaine by itself is not a specific marker of yeast source, and that a reference population of authentic products, with the betaine content of the declared ingredients, is needed before it is used as one. Species substitution (*C. utilis* or *Kluyveromyces* for *Saccharomyces*) alters the amino acid and lipid profiles more subtly and would be addressed by the fingerprint model of Section 9.2 against a reference set of authentic products. None of these questions has, to the author's knowledge, been addressed by NMR in the published literature, and the proposed measurements are therefore presented as feasible rather than as validated.

10.5 Brand Identity, Counterfeit Detection and Litigation-Grade Evidence

The feature matrix of Table 6 shows that the five products examined here are separable from one another on qualitative ^{1}H evidence alone, and that separation rests on stable, formulation- and process-linked features (trehalose versus dextrin, sucrose and maltodextrin dosing, nucleotide depth, HVP markers) rather than on trace components. A counterfeit "Marmite" made from another manufacturer's yeast extract or from an HVP base would fail the identity test at the level of Table 6, and a diverted or relabeled genuine product would pass it. For identity testing to carry evidential weight, however, a single spectrum of a single lot is not sufficient. The elements of a defensible identity method are a reference library of authentic product spectra spanning multiple lots and, ideally, multiple years of production; a documented sample preparation with buffering, internal standard and loading fixed; a fixed acquisition protocol with replicate acquisitions;

a similarity metric (spectral correlation coefficient, or a SIMCA class model with defined confidence limits) whose performance has been characterized on known positives and negatives; and a chain of custody for the questioned sample. These are the same elements that underpin the use of NMR fingerprinting in the fruit-juice and honey sectors and that have been reviewed in the context of food fraud generally.[10,62] The PNA laboratory's pursuit of ISO/IEC 17025 accreditation for its qNMR methods is directly relevant here, because an identity opinion offered in a commercial dispute will be examined first on the validation of the method and second on the data.

11. A Proposed PNA Analytical Program for Yeast-Extract Spreads

The data set of Section 8 was acquired as an exploratory survey. To convert it into a quality-control and authentication capability, the following program is proposed; it is written as a protocol outline so that it can be adopted directly into a laboratory method.

Sample preparation. Approximately 100 mg of paste, weighed to 0.1 mg, is dissolved in 1.00 mL of D_2O containing 0.15 M potassium phosphate buffer (pH 7.0 ± 0.05 in D_2O, measured as pD − 0.4), 0.5 mM DSS as reference and internal standard, and 0.1 mM EDTA-d_{12} (or unlabeled EDTA, with its signals excluded from analysis) to sequester paramagnetic and divalent ions. The solution is vortexed, centrifuged (10,000 g, 5 min), and 600 µL is transferred to a 5 mm tube. Dry matter is determined on a separate aliquot by oven drying or Karl Fischer titration so that results can be reported on both wet and dry bases.

^{1}H acquisition at 300 MHz. A NOESY-presaturation or excitation-sculpting water suppression sequence is used with a calibrated 90° pulse (recalibrated on a representative high-salt sample), a spectral width of 20 ppm, an acquisition time of 3.4 s, a relaxation delay of 25 s for the quantitative experiment (or 5 s for the fingerprint experiment), 64 to 128 transients and a probe temperature of 298.0 K. Processing uses 0.3 Hz exponential weighting, zero filling to 128k points, manual phasing and a polynomial or Whittaker baseline correction validated not to distort the broad glucan envelope. Integration of the analytes of Table 15 is against the DSS methyl signal, with deconvolution where Table 15 requires it.

Supporting experiments. A multiplicity-edited HSQC (2 to 4 h) is acquired for each new product or for each out-of-trend lot and is used for the quantitation of glutamate, lactate and glutathione; an HMBC is acquired once per product for assignment; a ^{31}P spectrum (inverse-gated decoupling, 30 s relaxation delay, 512 transients) is acquired for nucleotide speciation and for I+G verification; a DOSY or a CPMG-filtered ^{1}H spectrum is acquired where the peptide-to-free-amino-acid ratio or the dextrin molecular-mass distribution is at issue; and a TOCSY is acquired where individual branched-chain amino acids must be resolved.

Benchtop ^{1}H acquisition at 60 MHz. The same buffered solution is measured on the 4IR Solutions AI-60 with a water-suppression sequence, 32 to 64 transients and a 10 s relaxation delay, and the spectrum is bucketed at 0.04 ppm for the fingerprint model. The 60 MHz spectrum is validated against the 300 MHz spectrum of the same solution for the analytes marked feasible at 60 MHz in Table 15.

Chemometrics. The bucketed 300 MHz and 60 MHz fingerprints are mean-centered and Pareto-scaled; PCA is used for exploration and for T^2/Q control charts, SIMCA for class-membership (identity) decisions, and PLS regression, where an external reference value exists (for example Kjeldahl protein, HPLC glutamate or HPLC nucleotides), for calibration transfer. Cross-validation is performed with lot-wise (not spectrum-wise) partitioning, because replicate spectra of the same lot are not independent, and the number of latent variables is chosen by permutation testing rather than by minimum cross-validation error alone.[93,94] All model inputs and outputs are archived in a workbook that accompanies the report.

Reference library. Authentic retail lots of each product are acquired at intervals (quarterly is suggested) over at least two years, so that seasonal and raw-material variation is represented before the identity model is used for decisions. The present five spectra, one lot per product, constitute the first entries.

Reporting. Results are reported as concentrations (mmol/kg and g/100 g, wet and dry basis) with expanded uncertainties, as the process ratios of Section 9.3, and as the fingerprint distances (T^2, Q, or SIMCA class distance) with their control limits, together with the assignment key and the raw spectra as supporting data. The sections of this white paper that describe the PNA spectra are reserved for replacement by integrated, replicated results in a later version once the raw free induction decays have been reprocessed under the protocol above.

12. Critical Assessment and Limitations

The present data set is a survey of five products, one lot of each, acquired without buffering and interpreted, in Sections 8.3 to 8.9, from figures and, in Sections 8.10 to 8.15, from the processed spectra and not from the raw time-domain data, and the conclusions drawn from it must be sized accordingly. The following limitations are stated explicitly so that they are not overlooked when the material is reused.

Statistical scope. With one lot per product, nothing can be said about within-product variability, and the product differences described in Section 8 could in principle include a lot effect. The identity and consistency claims of Sections 9 and 10 are therefore claims about what the method can do once a reference population exists, not claims that have been demonstrated on this data set. The feature matrix of Table 6 is ordinal and visual and should not be reproduced as quantitative.

pH and referencing. The samples were dissolved in unbuffered D_2O. The displacement of the succinate singlet from 2.41 to 2.47 ppm in three of the five products, and its position at 2.42 ppm in Vegemite, show that the solutions differed in pH by roughly one unit, and the same displacement affects glutamate, glutamine, citrate, aspartate, the histidine ring and the nucleobases. Several of the tentative assignments in Section 8 — notably the 2.14 ppm singlet and the fine structure of the 2.3 to 2.5 ppm region — would be settled by a buffered re-acquisition. The ^{13}C axes of the different spectra are not on a common scale even in the shift-corrected figure set: measured on the numerical data, they read between 0.04 ppm above and 5.05 ppm below the DSS scale (Table 8), so that any comparison of ^{13}C positions between spectra requires those corrections to be applied. A single internal ^{13}C reference (DSS, or dioxane at 67.19 ppm) in every sample would remove this nuisance in the re-acquisition.

Dynamic range. In the New Zealand product and in Promite the sucrose signals are one to two orders of magnitude more intense than the amino acid signals of interest. At 300 MHz with a 16-bit digitizer this compresses the minor signals toward the noise; quantitation of the yeast-derived fraction of such products should use HSQC, ^{13}C or a selective-suppression ^{1}H experiment, and this has not yet been done.

Overlap at 300 MHz. The 3.5 to 4.0 ppm envelope, the 2.0 to 2.5 ppm region and the 0.9 to 1.05 ppm methyl cluster are not resolved at 7.05 T into their individual constituents. The consequences for quantitation are real and have been quantified for comparable media in the literature; the proposed remedies (deconvolution, HSQC quantitation, TOCSY assignment, higher field for method development) are standard but add time.[60] The 2D J-resolved spectrum of Marmite XO shows the strong-coupling artifacts that are inherent at this field.

Tentative assignments. The numerical analysis of version 1.2 has changed the standing of several of the assignments that were listed here as tentative in version 1.1. The choline-type singlet is resolved into glycerophosphocholine, free choline and — in the two British products, where version 1.1 had excluded it — betaine; the levulinate assignment in Promite now rests on three one-bond correlations and three ^{13}C lines and is graded probable, the ketone carbon not having been detected, so that spiking is still required; the olefinic cross-peak of Vegemite is supported by the allylic and chain methylene correlations; and the trehalose assignment is supported in all four yeast products by the one-bond and long-range correlations and, where a ^{13}C spectrum exists, by the ^{13}C set. Others remain where they stood: the niacin assignment rests on a weak singlet at 8.94 ppm, for which no two-dimensional correlation was found; the individual purine assignments rest on a few long-range correlations and on shift comparison with database values at

an uncertain pH, the purine ring carbons being invisible to the edited gHSQC; and 2,3-butanediol, the 5′-nucleotides, citrate in the New Zealand Marmite and sucrose in standard Marmite are tentative (Table 12). None of the assignments, trehalose and betaine included, has been confirmed by spiking, and each should be so confirmed before it is cited as fact. The mechanistic account of the trehalose difference between the British and Australasian products in Section 8.9 (yeast condition at intake and trehalase inactivation) is a pair of hypotheses consistent with yeast physiology, not a demonstrated result; the sensory reasoning about sweetness, salt and bitterness rests on the published taste-interaction literature and on the declared formulations, not on sensory data for these products.

The assignments added in version 1.2 carry limitations of their own. They were made on the spectra as processed and stored in MestReNova, recovered from a compressed representation; the compression error is below the noise, but the F1 resolution of the heteronuclear experiments (64 to 256 increments) limits carbon positions to ±0.3 to 1 ppm, and the ^{13}C axes had to be placed on a common scale by reference to internal resonances, since no carbon reference had been set consistently at acquisition. The solutions were unbuffered, at an apparent pH near 5, so that the titratable resonances depart from the neutral-pH database values and the comparison rests in those cases on the direction and size of the expected displacement. Reference shifts for pyroglutamate, glycerophosphocholine, levulinate and neutral tyrosine were taken from the Human Metabolome Database and those for the glucan and mannan residues from the literature, without re-measurement on authentic materials in this laboratory; none of the assignments has been confirmed by spiking. The multiplicity-edited gHSQC is blind to the purine and imidazole ring carbons and nearly so to formate, and the gHMBC F1 window stops short of the ketone region. The intensity ratios quoted are of three kinds and of unequal reliability. Those taken from the ^{1}H spectra (trehalose, sucrose and the α-anomer of glucose relative to alanine or to one another) were measured on spectra acquired with 90° pulses and a recycle time of 8.3 s, which should allow nearly complete relaxation of the signals compared, although T_1 was not measured. They are limited by overlap and, above all, by the treatment of the local baseline beneath a sharp doublet that stands on broader signals: the two treatments adopted (Lorentzian lines on a straight baseline, and direct integration between matched limits) differ by 7 to 15 percent, a curved baseline raises the result by up to a further third, and an uncertainty of about 10 percent is attached to the sharp doublets and of 20 percent or more to the glucan envelopes. The measurements were made numerically on the spectra as processed, not by integration or line fitting in MestReNova, and should be repeated there. Alanine, moreover, serves in these ratios as a ruler and not as a constant: a ratio to alanine compares two products only in so far as their free alanine is comparable. Those taken from ^{13}C line heights (the degree of inversion, pyroglutamate relative to glutamate) compare like carbons in spectra that were not acquired quantitatively and are good to perhaps 30 percent. Those taken from gHSQC cross-peak volumes (the branch proportion) compare like signals only, because the volumes were shown to misrepresent ratios between unlike signals by factors of up to three. All are ratios within single lots, and none replaces a qNMR measurement against an internal standard. The grading of Table 12 is uneven between products because the number of experiments available is uneven. And the two-dimensional data of Vegemite were acquired on a dissolution made two years after its ^{1}H spectrum. The remedies are those already listed: buffered re-acquisition with an internal ^{13}C reference, replicate lots, a non-edited or broadband-compensated HSQC, an HMBC window extended to 220 ppm, TOCSY, ^{31}P NMR and spiking with authentic standards.

Matrix effects. The high salt content of the pastes affects probe tuning and pulse calibration and reduces sensitivity; the iron fortification of the New Zealand product may affect relaxation; and the paste matrix is heterogeneous at the gram scale and must be homogenized before sub-sampling. None of these has been characterized in the present data set.

What NMR cannot do. NMR cannot distinguish added from native glutamate, cannot detect the aroma volatiles that define the sensory character of the products, cannot quantify the vitamins other than niacin at their fortification levels in a routine spectrum, cannot see potassium chloride or sodium chloride in ^{1}H, and cannot detect the biogenic amines at their relevant levels in the presence of the parent amino acids. For

these attributes the conventional methods of Table 3 remain necessary, and the correct position for NMR is as the integrating measurement that sits alongside them, not as their replacement.

Notwithstanding these limitations, the data set establishes that a single ^{1}H spectrum at 300 MHz separates the five products by class and by formulation, that the 2D and ^{13}C experiments confirm the principal assignments, that the nucleoside, trehalose, glucan, sucrose, trimethylammonium and organic-acid signals constitute a compact and interpretable set of quality and identity markers, and that the acid-hydrolysis markers distinguish an HVP-based product from a yeast-extract product at a glance. That is a sufficient basis on which to build the quantitative program of Section 11.

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Summary

This white paper has assembled the historical, technological, chemical and regulatory background of yeast-extract spreads and has used a set of Process NMR Associates spectra on five commercial products to demonstrate what multinuclear NMR contributes to their characterization. The products are the descendants of Liebig's observation that spent brewer's yeast can be concentrated into a savory food; Marmite has been made from Burton brewery yeast since 1902, Vegemite from Melbourne brewery yeast since 1923, and the New Zealand Marmite in Christchurch for most of a century, while Promite has evolved into a vegetable-protein-based product in which yeast extract is a minor component. The manufacturing chemistry — salt-induced autolysis or enzymatic hydrolysis, nuclease action on RNA, separation of the cell walls, evaporation and Maillard flavor development — determines the composition, and the composition of commercial extracts varies over ranges wide enough that "yeast extract" must be treated as a family of products rather than a substance.

NMR is the natural integrating measurement for such a matrix. In a single ^{1}H spectrum at 300 MHz the free amino acids, the RNA degradation products, the reserve and formulation carbohydrates, the organic acids and the choline compounds are all observed, and the ^{13}C, DEPT, HSQC, HMBC and J-resolved experiments confirm the assignments. The PNA spectra show that Marmite and Marmite XO carry an α-(1→4)/(1→6)-glucan residue from the brewery yeast and a rich inventory of RNA degradation products, largely nucleosides, that deepens with the 28-day maturation; that Vegemite is a trehalose-rich autolysate; that the New Zealand product is the same class of material dominated by added sucrose; and that Promite lacks, in ^{1}H, ^{13}C and HSQC alike, the RNA degradation products that any yeast extract must contain and carries instead the formate and levulinate markers of acid-hydrolyzed vegetable protein. The British and Australasian products are separated by the fate of the yeast's reserve carbohydrates and by the depth of nucleolysis: Marmite consumes most of its trehalose and retains glucan through a long salt-driven maturation and reaches palatability through salt and glutamate umami (a contribution of nucleotide synergy remaining to be demonstrated), whereas Vegemite retains trehalose — a yeast metabolite, not an added ingredient — and the New Zealand product and Promite add sucrose, in both cases moderating bitterness and saltiness and, where potassium chloride is used as a salt substitute, masking its off-notes. No single conventional specification parameter would deliver these conclusions.

In version 1.2 the two-dimensional spectra were recovered numerically from the MestReNova documents and assigned in full. One hundred and fifty-five one-bond and eighty-three long-range correlations and some thirty J-resolved multiplets were assigned, and fifty constituents or structural classes were graded for each product. The analysis establishes eighteen free amino acids in the British products, with pyroglutamate comparable in amount with glutamate, 4-aminobutyrate, and a population of small peptides; identifies glycerophosphocholine as the principal trimethylammonium compound, with free choline at about one-third of it in all four yeast products and betaine beside them in the two British products only; shows glycerol to be the most intense carbon signal of Marmite XO; characterizes the soluble α-glucan as a branched dextrin with between one residue in eight and one in five at a branch point, and detects cell-wall mannan beside it; finds trehalose in all four yeast products and shows, from the ^{1}H spectra, that the New Zealand Marmite is a trehalose-rich, glucan-poor extract of the Vegemite type (about 0.7 and 1.2 mol of trehalose per mol of free alanine, against 0.29 in Marmite and 0.08 in Marmite XO), in which about one-eighth of the added sucrose has been inverted; shows the RNA-derived material of the British products to be present largely as nucleosides and reassigns the 8.6 ppm singlet to histidine; detects monounsaturated fatty acyl chains in Vegemite alone; and supports the levulinate assignment in Promite with three one-bond correlations, beside citrate and formate and the absence of asparagine, tryptophan, trehalose, glycerophosphocholine and uridine. Several readings of version 1.1 were corrected in consequence, among them the scale of the ^{13}C axes, the trehalose content of the New Zealand product and the inference of a nucleotide-based umami synergy in the British products. The gHSQC cross-peak volumes were shown by comparison with the ^{1}H spectra not to be quantitative between unlike signals, and the relative amounts quoted rest on the ^{1}H and ^{13}C spectra.

Building on those observations, a quantitative NMR analyte panel of some twenty constituents, a Pareto-scaled fingerprint control scheme with T^2/Q limits, a set of dimensionless process-diagnostic ratios (degree of hydrolysis, deamidation, trehalose/glucan, nucleotide speciation, lactate/acetate hygiene, GABA), and authentication strategies for carbohydrate dilution, HVP substitution, added 5′-ribonucleotides and yeast-source substitution have been proposed, together with a benchtop 60 MHz tier for routine consistency monitoring. Equally, the limits have been stated: NMR cannot distinguish native from added glutamate, cannot see the aroma volatiles, most of the vitamins, the inorganic salts or the biogenic amines at relevant levels, and the present survey — one unbuffered lot per product, without integration against an internal standard or replication — supports qualitative class and formulation conclusions but not statistical statements about lot variability. A protocol for buffered, internally referenced, replicated acquisition at 300 and 60 MHz, with ^{31}P speciation and a two-year reference library, has been set out so that a later version of this document can carry integrated, replicated results and a validated identity model.