"Milk on Ice": A detailed analysis of Ernest Shackleton's century-old whole milk powder in comparison with modern counterparts

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ABSTRACT

Whole milk powder (WMP) manufactured in New Zealand in 1907 was sent to the Antarctic continent with the Shackleton-led British Antarctic Expedition from 1907 to 1909. This powder was stored at ambient conditions at Shackleton's Hut at Cape Royds, Antarctica, for over 100 yr before a sample was collected on behalf of Fonterra by the Antarctic Heritage Trust. Having spent most of its existence both dried and in frozen storage, any deleterious reactions within the WMP would have been markedly retarded. The composition and some properties of the roller-dried Shackleton's WMP are reported along with those of 2 modern spray-dried New Zealand WMP. The Shackleton powder was less white and more yellow than the modern WMP and was composed of flakes rather than agglomerated particles, consistent with that expected of a roller-dried powder. Headspace analysis showed lipolytic and oxidative volatile compounds were present in the Shackleton WMP, indicting some deterioration of the milk either before powder manufacture or on storage of the finished product. On a moisture-free basis, the Shackleton WMP had higher protein, higher fat (with a markedly higher free fat level), higher ash, and a lower lactose level than the modern WMP. The lysine level was lower in the Shackleton WMP compared with the spray-dried powders, whereas the fatty acid composition was relatively similar. The sodium level was markedly higher in the Shackleton WMP compared with the spray-dried powder, which is probably due to the addition of an alkaline sodium salt to adjust the pH of the milk before roller drying. Lead, iron, and tin levels were markedly higher in the Shackleton WMP compared with the spray-dried powders, possibly due to the equipment used in powder manufacture and the tin-plated cases used for storage. The proteins in the Shackleton WMP were more lactosylated than in the spray-dried powders. The Shackleton WMP had a higher ratio of κ-casein A to

B variants and a higher ratio of β-lactoglobulin B to A variants than the spray-dried powders, whereas the α_{S1}-casein, β-casein, α_{S2}-casein, and α-lactalbumin protein variants were similar in all powders. The total phospholipid content was markedly lower in the Shackleton WMP than the spray-dried powders, primarily due to a lower phosphatidylethanolamine concentration. The molecular species distributions within the phospholipid classes were generally similar in the 3 powders. Claims are sometimes encountered that the milk of today is different from that consumed by previous generations. However, this comparative study has shown that the Shackleton WMP was generally similar to modern WMP. Although differences in some components and properties were observed, these were attributable to the manufacturing equipment and processes used in the pioneering years of WMP manufacture.

Key words: Ernest Shackleton, Antarctica, whole milk powder, spray dried, roller dried

INTRODUCTION

The recent discovery of the ship *Endurance* 3 km under the ice at the bottom of the Weddell Sea in Antarctica has reignited interest in Sir Ernest Shackleton and his ill-fated Imperial Trans-Antarctic expedition (Fountain, 2022a,b). The *Endurance* was trapped in the ice of the Weddell Sea in January 1915 and sank in November 1915. The crew survived on the ice of the Weddell Sea and then sailed in lifeboats reaching Elephant Island in April 1916. Shackleton, with a small 5-man crew, sailed 700 nautical miles (1,300 km) from Elephant Island to South Georgia. They then traversed the mountainous unexplored interior of the island to reach the whaling station at Stromness. After several attempts to return to Elephant Island were thwarted due to pack ice, the remaining crew was eventually rescued in August 1916, more than 19 mo after first being trapped on the ice. These adventures have been painstakingly reported in books by Sir Ernest Shackleton himself (Shackleton, 1920) and Alfred Lansing (Lansing, 1959).

Before the Imperial Trans-Antarctic expedition, and before the successful Roald Amundsen-led South Pole

expedition (1910–1912) and the ill-fated Robert Falcon Scott-led British Antarctic (*Terra Nova*) expedition (1910–1913), Shackleton led an attempt to reach the South Pole in the British Antarctic (*Nimrod*) expedition from 1907 to 1909. Although this expedition did not achieve its ultimate goal, falling 97.5 nautical miles (180 km) short of the South Pole, they had other remarkable achievements, including the first ascent of Mount Erebus, the estimated location of the South Magnetic Pole, and extensive geological and zoological studies of the Antarctic region. The base camp of the *Nimrod* expedition was established at Cape Royds, and a prefabricated hut was erected in 1908, which was used as winter quarters and to store equipment and supplies. The achievements of the *Nimrod* expedition attracted widespread interest and Ernest Shackleton received public honors including a knighthood. The exploits of this expedition were recorded in Ernest Shackleton's book entitled *The Heart of the Antarctic* (Shackleton, 1909).

In 2002, the Antarctic Heritage Trust launched the Ross Sea Heritage Restoration project to preserve the 4 significant Antarctic huts and their extensive artifact collections. During the conservation and restoration activities of Shackleton's Cape Royds hut, several significant artifacts were uncovered, including cases of Mackinlay's whisky still wrapped in their paper and protective straw sheaths. The whisky underwent scientific analysis by the owner of the Mackinlay brand (Whyte & Mackay) and the Scotch Whisky Research Institute (Pryde et al., 2011). From this research, the unique Shackleton whisky was recreated and marketed. In addition to whisky, brandy, and other artifacts, a container of Defiance brand dried milk, a milk powder product of New Zealand origin, was discovered (Figure 1A and 1C). The powder used the Defiance brand name for the Imperial Dry Milk Company Ltd. of the UK but was marketed as a Glaxo product when this brand was registered by Joseph Nathan and Company in 1906.

The *Nimrod* departed Lyttleton Harbour, New Zealand, for Antarctica on January 1, 1908. An examination of the inventory listing of the *Nimrod's* main food supplies includes 1,000 pounds (454 kg) of Glaxo milk powder, and it is later revealed that "Messrs. Nathan and Company, of Wellington, presented the expedition with sixty-eight cases of 'Glaxo' dried milk, and this preparation, which consists of the solid constituents of fresh milk, was a valuable addition to our food-stuffs. The same firm presented us with 192 lb. of New Zealand butter and 2 cases of New Zealand cheese" (Shackleton, 1909, pp. 10–11). The Defiance brand dried milk, with its distinctive black rooster logo (Figure 1B), had its beginnings in Bunnythorpe near Palmerston North in the Manawatu region of New Zealand, and was the first

dried milk manufacturer in New Zealand, commencing operations in 1904. The factory was destroyed by fire in 1906 and was rebuilt. However, when the reconstruction was nearly complete in mid-1906, the boiler was destroyed by a rival dairy factory owner through a gelignite explosion. Although arrested for his role in the explosions and suspected of starting the fires that destroyed the original factory, a local jury acquitted this rival dairy factory owner. These events occurred at around the time the Shackleton cases of the Defiance brand dried milk were produced as these are likely to be from 1907 based on the sailing schedule of the *Nimrod* (Davenport-Hines, 1992; Wynyard, 2016).

The Defiance brand dried milk was manufactured by a roller-drying process likely to be similar to that patented by Just (1902). The rights for this roller-drying process were later sold to James Robert Hatmaker, with the method later called the Just-Hatmaker process after Hatmaker's company made some improvements to the original process (Hatmaker, 1908; Merrett, 1908). As this was before the use of vacuum-assisted evaporation, the milk may have been partially concentrated by vigorous boiling before being poured between 2 steam-heated revolving cylinders so that the water rapidly evaporated leaving a thin sheet of dried milk that was scraped from the rollers by blades before a full rotation of the cylinders. The resultant dried milk was milled and sieved to give a dried milk powder. Those early milk powders suffered from solubility issues, producing satisfactory milk when reconstituted in hot water but separating into sediment and whey when the milk was cooled. It took several years to refine the process to provide an adequately soluble milk powder. Thus, the Shackleton whole milk powder (**WMP**) is likely to be from the factory at a time when some solubility issues persisted (Davenport-Hines, 1992).

With the facilitation of the Antarctic Heritage Trust, a few hundred grams of the Defiance brand dried milk were subsampled from the original container in Shackleton's Cape Royds hut for the Fonterra Research and Development Centre to analyze. This Shackleton dried milk is possibly the best-preserved sample manufactured during the pioneering years of commercial milk powder production. It allows for a compositional and microstructural comparison of the early roller-dried milk powder with modern spray-dried WMP. In this study we compared the major component composition, major and trace mineral composition, protein composition, fatty acid composition, phospholipid composition microstructural properties, color analysis, and volatile component analysis of the Shackleton milk powder with those from 2 modern commercial standard WMP samples. This allows a thorough examination of the similarities and differences between a roller-dried milk

Figure 1. (A) Tin-plated can of Defiance brand dried milk. The can had been pried open at the lid with inner foil seal out and was half-full of milk powder. The dimensions of the can are 490 × 335 × 335 mm (approximately 19 × 13 × 13 inches). Photo courtesy of the Antarctic Heritage Trust, Christchurch, New Zealand. (B) The Joseph Nathan & Sons Bunnythorpe Defiance Dried Milk Factory, circa 1904. Photo courtesy of Massey University Dairy Records Archive, Reference Number GB-4, Massey University, Palmerston North, New Zealand. (C) Label from the can of milk powder from Shackleton's Cape Royds hut. Photo courtesy of the Antarctic Heritage Trust, Christchurch, New Zealand.

powder manufactured over 100 years ago with the modern spray-dried counterparts.

MATERIALS AND METHODS

WMP Samples

The exact manufacturing conditions and storage history of the Defiance brand dried milk before reaching Antarctica are unknown. Once the milk powder arrived in Antarctica, the original container was likely to have been stored at the ambient conditions in or around Shackleton's Hut at Cape Royds, which is reported to have an indoor temperature for the year 2010 ranging from a minimum of −32.5°C during winter to a maximum of 3.3°C during summer (Pryde et al., 2011). In 2008, members of the Antarctic Heritage Trust facilitated the collection of a subsample of the milk powder into a 1-L polytetrafluoroethylene (**PTFE**) bottle (Cowie Technology Group Ltd., Middlesbrough,

UK) using a PTFE scoop after first using the scoop to push the topmost 2 to 3 cm of milk powder away from the center of the original tin-plated case to the outer edges, and then collecting milk powder from the center of the case, before sealing the PTFE bottle firmly with the air-tight screw-top lid. The subsample was kept frozen at −18°C until use. Before sampling for this study, an earlier sample had been collected in 2002 for a microbiological study where it was reported that the storage tin had already been opened (Ronimus et al., 2006). For comparative purposes, 2 modern-day commercial noninstantized spray-dried WMP samples were obtained from Fonterra Cooperative Group, New Zealand.

The Defiance brand dried milk was a roller-dried WMP obtained from Antarctica and will be referred to as "Shackleton WMP," whereas the 2 commercial spray-dried WMP will be referred to as "WMP1" and "WMP2." The first, WMP1, was manufactured at Fonterra's Darfield factory in the Canterbury region of the South Island of New Zealand (40 km west of Christchurch, and 619 km from Bunnythorpe) in late March (early autumn, 2021), whereas WMP2 was manufactured at Fonterra's Pahiatua factory (19 km east-southeast of Palmerston North, and 24 km southeast of the Bunnythorpe factory) in early October (mid spring, 2021). These 2 WMP samples were standardized for both protein content (by milk permeate addition) and fat content, which is common practice in modern commercial milk powder manufacture (Walstra et al., 1999; Bylund, 2015).

Color Measurements

The powder samples were placed in clear polypropylene containers with a diameter of 4 cm to a depth of 5 cm. White paper was wrapped around the sample container and the container was placed on a white paper sheet. The color was measured using a CR300 Chroma Meter (Konica Minolta Inc., Tokyo, Japan), using the D_{65} light source. The Chroma Meter was calibrated with the CR-A43 white calibration plate before use. The tip of the measuring head was placed firmly on the surface of the powder within the container and the measurement started. Three measurements on different areas of the powder surface were made for each sample. The measurement gave the color properties expressed in 3 variables: L* for white (+) to black (−), a* for red (+) to green (−), and b* for yellow (+) to blue (−). The whiteness index (**WI**), defined as a measure of how closely a sample compares to a perfect reflecting diffuser, was calculated using Equation 1, and the yellowness index (**YI**), defined as the level that the color

of a sample is shifted from white or colorless toward yellow, was calculated using Equation 2.

$$\text{WI} = 100 - \left[\left(100 - L* \right)^2 + \left(a* \right)^2 + \left(b* \right)^2 \right]^{0.5} \qquad [1]$$

$$\text{YI} = 142.86 \times \frac{b*}{L*} \qquad [2]$$

SDS-PAGE

Sodium dodecyl sulfate-PAGE was carried out under reducing conditions using the methods described previously (Anema and Klostermeyer, 1997; Havea, 2006). A 15% (wt/wt) solution of milk powder in sample buffer (0.5 *M* Tris/HCl buffer at pH 6.6 containing 2% SDS and 0.01% bromophenol blue) was first prepared to fully disperse the powder. The WMP1 and WMP2 samples were further diluted 1:40 with sample buffer, whereas the Shackleton WMP sample was diluted 1:40, 1:30, 1:20, and 1:10 with sample buffer. The samples were reduced by treatment with 2-mercaptoethanol (2% vol/vol) and then heated (98°C for 5 min) in a heating block. For all gel electrophoresis analyses, the protein samples were separated using precast Bolt 12%, Bis-Tris, 1.0 mm, mini protein gels, and the associated MES SDS running buffer (Thermo Fisher Scientific Inc., Waltham, MA). After electrophoresis separation, the gels were stained with amido black 10B (0.1% wt/vol in 10% aqueous acetic acid) and then de-stained (10% aqueous acetic acid) until a clear background was achieved. The gels were scanned using an Imagescanner III (GE Healthcare, Wauwatosa, WI).

Headspace Solid-Phase Microextraction and GC-MS

Shackleton WMP, WMP1, and WMP2 were all treated under the same conditions to allow qualitative comparisons to be made. Because of the poor dispersibility of Shackleton WMP in water, a headspace solid-phase microextraction (**HS-SPME**) technique was used to extract volatiles directly from dry samples of each milk powder. This approach meant that homogeneous distribution of any internal standard quantification would not be possible, and so quantification of the volatile compounds was not attempted. Each sample of milk powder (0.5 g) was placed in a Chromacol 20-mL glass vial (Thermo Fisher Scientific, Waltham, MA) and crimp-sealed with a Teflon-backed septum (Thermo Fisher Scientific, Waltham, MA). The headspace of each vial was extracted for 20 min at 70°C using a DVB/Carbon WR/PDMS SPME Arrow fiber (1.1 mm

outside diameter, 120 μm film thickness, 20 mm length; CTC Analytics, Zwingen, Switzerland).

Gas chromatography-mass spectrometry analysis of extracted volatiles was performed using a TQ8050NX GC-MS/MS instrument equipped with an AOC6000 autosampler (Shimadzu, Kyoto, Japan) and fitted with an SH-Stabilwax-DA capillary column (30 m × 0.25 mm × 0.25 μm stationary phase thickness; Shimadzu, Auckland New Zealand). Analytes were desorbed from the SPME fiber for 2 min at 250°C in the GC injection port, which contained a silanized SPME Arrow liner (1.7 mm i.d., Shimadzu). The splitless injection technique was used to transfer volatile compounds from the injection port to the capillary column. The column oven was held at 50°C for 3 min before the temperature was increased to 80°C at 3°C min^{-1}, then increased to 120°C at 5°C min^{-1}, before finally increasing to 250°C at 25°C min^{-1}, where the temperature was held for 16 min. Helium was used as the carrier gas with a column flow rate of 1.1 mL·min^{-1}, a purge flow of 3 mL·min^{-1}, and a split ratio of 10:1.

The MS interface was held at 250°C and the ion source at 200°C. The MS was operated in electron impact (EI) mode at 70 eV with an interval of 0.2 s. Masses were scanned from 45 to 350 m/z. Mass spectra for peaks of interest were tentatively identified by retention times and by comparison with library spectra from the Wiley compound library (Wiley, Edition 7; https://sciencesolutions.wiley.com/solutions/technique/gc-ms/).

Compositional Analysis

The moisture and ash of the powders were determined by thermogravimetric analysis using a LECO TGA701 analyzer (LECO Australia Pty Ltd., Castle Hill, NSW, Australia). The total nitrogen (**TN**) and NPN were measured using ISO 8968–4|IDF 20–4 (ISO, 2016) based on the Kjeldahl method (Kjeldahl, 1883). The protein was determined as TN × 6.38. The lactose and phosphate were determined by using a flow injection analyzer and the QuikChem 21–250.00–2-A method (Lachat Instruments, Loveland, CO). The fat content was determined by ISO 1211|IDF 001 (ISO, 2010) based on the Röse–Gottlieb method (Röse, 1888; Gottlieb, 1892); and free fat was determined gravimetrically after accelerated solvent extraction with diethyl ether. Total fatty acids were determined as FAME using ISO 15884|IDF 182 (ISO, 2002a) and ISO 15885|IDF 184 (ISO, 2002b). Citrate was determined as citric acid using the UV-enzymatic method for the determination of citric acid in foodstuffs and other materials (Boehringer Mannheim GmbH, Manheim, Germany). Chloride was determined by a potentiometric titration using ISO 21422|IDF 242 (ISO, 2018). Nitrate and nitrite were determined using the method ISO 14673–3|IDF 189–3 (ISO, 2004). All other mineral components were determined by inductively coupled plasma emission spectroscopy with mass spectrometry as required (method 984.27, AOAC International, 2023a).

AA Analysis

The tryptophan level in the milk powders was determined using method 2017.03 (AOAC International, 2023b) whereas the other amino acids were measured using method 2018.06 (AOAC International, 2023c).

Protein Characterization

Individual caseins and β-LG, and α-LA were characterized using reversed-phase ultra-high-performance liquid chromatography (**UHPLC**)–MS (biozen Intact XB-C8 column packed with 3.6-μm particles, 150 × 4.6 mm, Phenomenex). Samples were prepared in 6 M guanidine hydrochloride/0.1 M Bis-Tris buffer (pH 6.8), and reduced with dithiothreitol as described by Bobe et al. (1998). Mass spectrometry analysis was conducted using 6500QT mass spectrometer (SCIEX) in positive MS mode, with an ion-spray voltage of 4,000 V, spray temperature at 600°C, ion source gas 1 and 2 at 80 and 20 (arbitrary units) respectively, and a curtain gas of 20 (arbitrary units). The declustering and entrance potentials were 135 and 10 V, respectively. Protein mass deconvolution and analysis was carried out using SCIEX OS-MQ software (V 2.1.6.59781). Observed masses were compared with the reported literature values (Farrell et al., 2004)

Phospholipid and Glycosphingolipid Analysis

Phospholipids and glycosphingolipids were extracted as previously described (Fong et al., 2013; Ma et al., 2020). Briefly, each powder sample was rehydrated into a solution with approximately 10% TS. An aliquot of 0.5 mL of each solution was extracted with 4 mL of chloroform/methanol (1:2). After phase partition, the bottom phase was made to 5 mL with chloroform/methanol (1:2) before liquid chromatography-MS analysis.

Five microliters of each extract were injected by the Vanquish UHPLC system and analyzed by a TSQ Altis mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Individual classes of phospholipids were separated on an APS-2 Hypersil column packed with 3-μm particles (150 mm × 2.1 mm, Thermo Fisher Scientific, Waltham, MA) and calibrated against purified natural standards: bovine phosphatidylethanolamine (**PE**), bovine phosphatidylcholine (**PC**), porcine phosphatidyl-

serine (**PS**), bovine phosphatidylinositol (**PI**) and bovine sphingomyelin (**SM**) obtained from Avanti Polar Lipid (Alabaster, AL). Whereas the separation of hexosylceramide (**HexCer**) and lactosylceramide (**LacCer**) were achieved on a Luna hydrophilic interaction liquid chromatography (**HILIC**) column packed with 5-µm particles (250 mm × 4.6 mm, Phenomenex, Torrance, CA) and they were calibrated using standards purified from bovine buttermilk (Matreya, Pleasant Gap, PA).

Microstructural Analysis

Confocal Microstructural Analysis. In confocal laser scanning microscopy (**CLSM**), contrast is obtained by differences in fluorescence. The fluorescent dyes, Nile Red (0.5%, Sigma-Aldrich, Auckland, New Zealand) and Fast Green FCF (0.2%, Sigma-Aldrich, Auckland, New Zealand), were made up in polyethylene glycol (**PEG**). Milk powder samples were placed on concave microscope slides; approximately 10 µL of the dual dye was added to a coverslip which was then placed over the powder samples. The slides were then examined at room temperature with the ZEISS LSM 800 Airyscan confocal microscope (Carl Zeiss, Oberkom, Germany) using the 40× and 63× oil immersion objectives. Laser excitation parameters were set at 488 nm (argon laser) and 633 nm (helium-neon laser) for the Nile Red and the Fast Green probes, respectively. Randomly selected areas of each sample were imaged; the micrographs produced were either combined or separated into images of the fat (red) or protein (green) phases.

Scanning Electron Microscopy. A few grams of the loose milk powder samples were attached (separately) to a double-sided adhesive carbon tab mounted on a scanning electron microscopy stub and then examined with a Hitachi benchtop SEM TM4000Plus scanning electron microscope (Hitachi High-Tech, Tokyo, Japan).

Transmission Electron Microscopy and Light Microscopy. The preparation of dairy powders for transmission electron microscopy (**TEM**) involved 4 stages: fixation, dehydration, embedding, and sectioning. Small amounts of loose milk powders were added to low-temperature-gelling agarose and allowed to set. Once set, the agarose-embedded powder samples were cut into cubes of ~3 mm and put in Bijou bottles containing 2.5% glutaraldehyde in 0.2 *M* sodium cacodylate buffer (pH 7.2). This was kept at 5°C for 24 h. The samples were then postfixed in 1% osmium tetroxide in phosphate buffer overnight at 4°C, dehydrated in graded concentrations of acetone, and embedded in a resin made from Procure 812 (ProSciTech Pty Ltd., Australia), methyl-5-norbornene-2,3-dicarboxylic anhydride (ThermoFisher Scientific Inc., Fair Lawn, NJ),

dodecenyl succinic anhydride and benzyldimethylamine (Ted Pella Inc., Redding, CA). Ultra-thin sections (80 to 90 nm thickness) were cut using a diamond knife and collected on 300 mesh copper grids before staining with uranyl acetate and lead citrate. Sections were examined and photographed with a JEM-1400 Flash transmission electron microscope (JEOL Ltd., Tokyo, Japan) at 100 kV.

RESULTS AND DISCUSSION

Visual Appearance, Microstructural Analysis, and Color Measurements

Images of the milk powders are shown in Figure 2A. Both WMP1 and WMP2 were very fine powders, whereas the Shackleton WMP was coarse and more granular in comparison, though still quite fluffy in appearance despite the coarseness of the particles. The roller-dried Shackleton WMP was somewhat more yellow in color than the spray-dried milk powders. Considering the roller-drying manufacturing process, the granular nature of Shackleton WMP will be a consequence of the milling and sieving of the film of dried powder compared with spray-drying for WMP1 and WMP2. Similarly, the more intense yellow color of the Shackleton WMP will be a consequence of Maillard browning reactions resulting from the prolonged and more intense heat treatment used in roller-drying (Davenport-Hines, 1992) when compared with the processes used to make spray-dried milk powder. These Maillard reactions may have continued, albeit slowly, during the very long storage period of the Shackleton WMP.

The milk powder samples were analyzed using scanning electron microscopy, and typical micrographs of WMP1 and WMP2 and the Shackleton WMP are shown in Figure 2B. As expected, the spray-dried WMP1 and WMP2 and the roller-dried Shackleton WMP displayed markedly different powder particle morphologies. The microstructure of WMP1 and WMP2 showed spherical particles agglomerated together to form irregular powder particles of clustered spheres ranging in size from about 50 to 400 µm. The observed microstructure of WMP1 and WMP2 was typical of that observed for agglomerated spray-dried WMP (Caric and Kaláb, 1987; McKenna et al., 1999; Murrieta-Pazos et al., 2011). In contrast, the Shackleton WMP was composed of compact, irregularly shaped, thin flakes with sharp edges. The sizes of the flakes ranged from about 50 to 300 µm. The surface of the flakes was rough with distinct channels, possibly a consequence of evaporating water from the milk film against the heated rollers. This microstructure is consistent with that expected of a roller-dried WMP where a film of milk is dried on
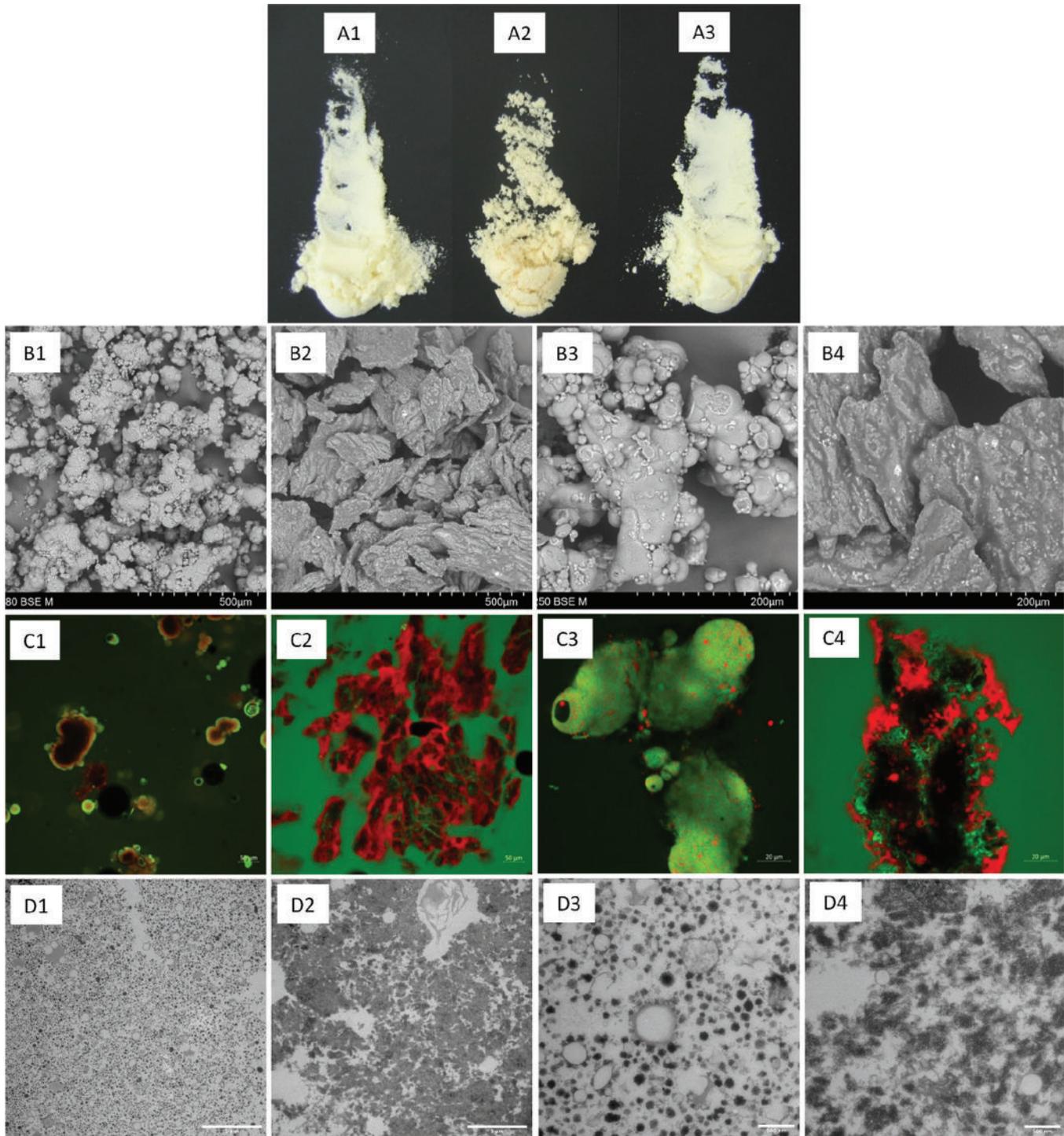
Figure 2. (A) Images of samples of the whole milk powders (WMP). (A1) WMP1. (A2) Shackleton WMP. (A3) WMP2. (B) Scanning electron microscopy images of WMP samples. (B1) WMP1 at 80× magnification. (B2) Shackleton WMP at 80× magnification. (B3) WMP1 at 250× magnification. (B4) Shackleton WMP at 250× magnification. (C) Confocal laser scanning microscopy images of WMP samples. Red = fat, bright green = protein, pale green/black = background. (C1) WMP1 at 10× magnification. (C2) Shackleton WMP at 10× magnification. (C3) WMP1 at 63× magnification. (C4) Shackleton WMP at 63× magnification. (D) Transmission electron microscopy images of WMP samples. (D1) WMP1 at 1,500× magnification. (D2) Shackleton WMP at 1,500× magnification. (D3) WMP1 at 10,000× magnification. (D4) Shackleton WMP at 10,000× magnification. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

Table 1. Color parameters of whole milk powders (WMP); the results represent the average and SD of 3 repeat measurements[1]

Sample[2]	L*	a*	b*	Whiteness index	Yellowness index
Shackleton WMP	86.9 ± 0.3^{c}	-2.1 ± 0.1^{a}	22.8 ± 0.2^{a}	73.7 ± 0.3^{b}	37.4 ± 0.5^{a}
WMP1	93.3 ± 0.1^{b}	-5.6 ± 0.2^{c}	21.0 ± 1.3^{b}	77.3 ± 1.3^{a}	32.1 ± 2.0^{b}
WMP2	93.9 ± 0.1^{a}	-4.8 ± 0.0^{b}	20.2 ± 0.2^{b}	78.3 ± 0.2^{a}	30.8 ± 0.3^{b}

[a–c]Different superscript letters in same column indicate a significant difference ($P < 0.05$).

[1]The measurement gave the color properties expressed in 3 variables: L* for white (+) to black (−), a* for red (+) to green (−), and b* for yellow (+) to blue (−).

[2]Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

heated rollers and subsequently milled and sieved to form the powder particles (Palmer and Dahle, 1922; Caric and Kaláb, 1987).

Confocal laser scanning microscopy of the powder particles also revealed distinct differences between WMP1, WMP2, and the Shackleton WMP (Figure 2C). The micrographs of WMP1 and WMP2 showed powder particles with small (1–2 μm) spherical fat globules dispersed uniformly within the amorphous protein/lactose phase of the powder particles. There were a few larger areas of coalesced fat at the surface of the particles; however, these were still only a few micrometers in size. These CLSM micrographs are consistent with those observed previously for spray-dried WMP particles (McKenna et al., 1999; Ye et al., 2007; Hazlett et al., 2021). In contrast, the Shackleton WMP did not reveal any distinct fat globules. The fat was coalesced into large amorphous areas that appeared to be separate from the amorphous protein-lactose phase. The areas of fat were extensive and large with dimensions up to hundreds of micrometers. It appears that the roller-drying process caused the extensive disruption of the fat globules, and these CLSM images are consistent with the observation the roller-dried WMP have extremely high free fat levels, often exceeding 90% of the available fat (Koc et al., 2003; Courtois, 2013).

Transmission electron microscopy allowed the protein material in the milk powder samples to be analyzed at a high magnification and therefore, detail on the casein micelle structure could be visualized. Typical TEM micrographs of WMP1, WMP2, and the Shackleton WMP are shown in Figure 2D. The sample preparation process for the TEM images removes the fat from the samples leaving roughly spherical voids representing the fat globules and irregular voids for the free fat. The latter are difficult to distinguish from the amorphous lactose phase of the powder particles.

The TEM microstructure of WMP1 and WMP2 showed spherical casein micelles ranging in size from about 10 nm to about 200 nm uniformly dispersed throughout the particle, presumably in the amorphous lactose phase of the dried milk powder. In addition, there are larger roughly spherical particles from the fat globules with their surfaces partially composed of casein micelles, as expected for a homogenized milk sample. The observed TEM microstructures for WMP1 and WMP2 are similar to those observed previously for agglomerated spray-dried WMP (McKenna et al., 1999). In contrast, the Shackleton WMP was composed of fused casein particles, with large void areas presumably from the free fat (clearly observed in the confocal micrographs; Figure 2C2 and 2C4). Higher magnification showed that the protein network was composed of roughly spherical particles and filamentous protein aggregates interlinked into a network structure. This aggregated arrangement of the protein in the Shackleton WMP, along with the high levels of free fat would account for the poor solubility of this milk powder.

The color properties of the milk powders (Table 1) showed that the L* of the powders were highly positive, indicating a light color. The L* of the Shackleton WMP was appreciably lower than that of WMP1 (indicating a darker color), which in turn had a lower L* than WMP2. The a* variable showed all powders were slightly toward the green hue, with WMP1 having a lower (thus greener hue) than WMP2, which in turn had a lower a* than the Shackleton WMP. The b*, a measure of the yellow/blue color was positive indicating a yellow color, with the Shackleton WMP having a higher b* (more yellow) than WMP1 and WMP2, which were similar to each other. The calculated WI was appreciably lower and the YI higher for the Shackleton WM when compared with WMP1 and WMP2.

Electrophoresis Analysis by SDS-PAGE

The electrophoresis patterns for the Shackleton WMP, WMP1, and WMP2 are shown in Figure 3. Similar patterns were observed for WMP1 and WMP2 with the 4 major casein proteins, β-LG, and α-LA clearly resolved, as well as clear bands for high molecular weight proteins, which include BSA, immunoglobu-
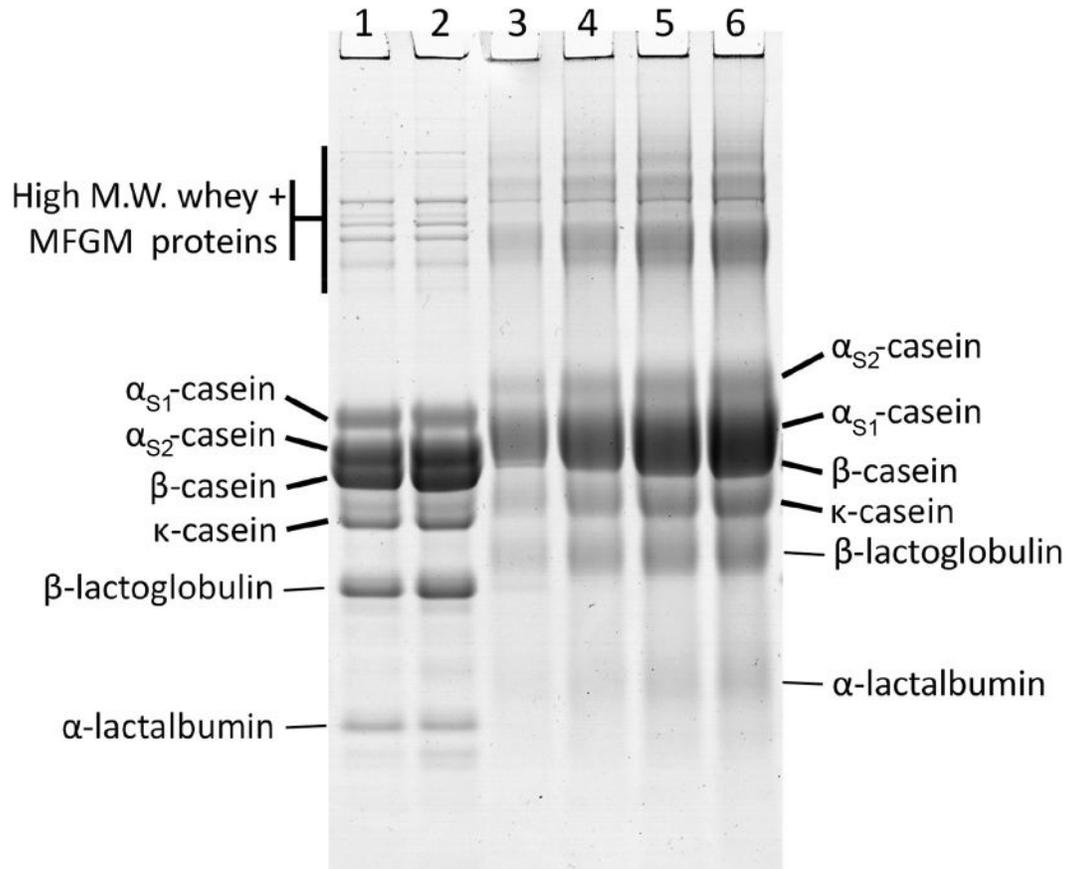
Figure 3. Sodium dodecyl sulfate-PAGE patterns of whole milk powder (WMP) samples. Milk powders were initially reconstituted to 15% wt/wt with sample buffer before further dilution. Lane 1: WMP1 diluted 1:40 with sample buffer. Lane 2: WMP2 diluted 1:40 with sample buffer. Lanes 3 to 6: Shackleton WMP diluted 1:40, 1:30, 1:20, and 1:10, respectively, with sample buffer. High M.W. whey refers to high-molecular-weight whey proteins. MFGM refers to milk fat globule membrane proteins. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

lins, lactoferrin, and proteins from the milkfat globule membrane. For the Shackleton WMP, the bands were blurred and diffuse, which is a known phenomenon for aged powders due to lactosylation of protein (Le et al., 2011); however, bands could still be attributed to the (modified) caseins, β-LG, and α-LA. These had not traveled as far down the gel, indicating a higher molecular weight, which was expected due to Maillard reactions causing lactose to bind with the proteins. The α_{S1}-CN and β-CN were not resolved into separate bands, and considerably blurred high molecular weight bands were observed, which may be due to cross-linked proteins.

Qualitative Volatile Profile by HS-SPME and GC-MS

Illustrative examples of the qualitative selected ion chromatographs for carboxylic acid, aldehyde and alcohol, and hydrocarbon volatile profiles of Shackleton WMP, WMP1, and WMP2 are shown in Figure 4. Substantial differences were evident in Shackleton WMP versus WMP1 and WMP2 samples, with a gas chromatographic profile for the Shackleton WMP being indicative of a WMP with substantial lipolytic and oxidative off-flavors.

The simplest explanation for the presence of most of these compounds is that, despite favorable storage conditions, Shackleton WMP has gone somewhat past its best-before date for product quality (no date marking was evident from the label shown in Figure 1C, although the probable manufacture date was sometime around 1907). When WMP is packed under air, the sensory quality is now known to deteriorate due to lipid oxidation at a rate that has a U-shaped dependency upon the water activity (a_w), with a minimum rate of oxidation occurring with a_w in the range of 0.2 to 0.4
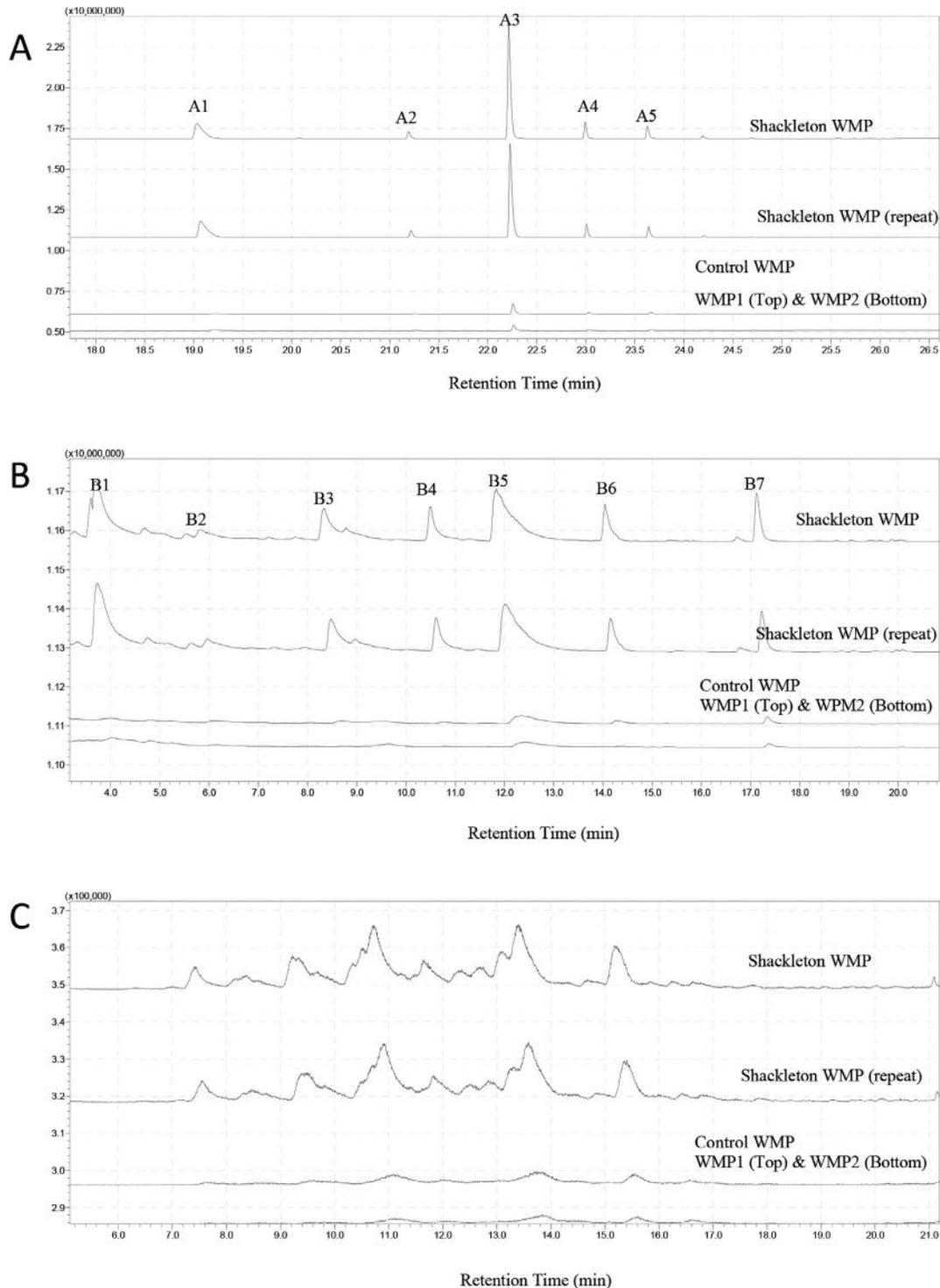
Figure 4. Illustrative examples of substantial differences between Shackleton whole milk powder (WMP), WMP1, and WMP2 as seen in qualitative GC-MS chromatograms. Selected ion chromatograms are shown for $m/z = 60$, 56, and 119 in panels A, B, and C, respectively. Regions of the chromatograms were selected to illustrate differences in classes of compounds, namely carboxylic acids, aldehydes (and alcohols), and unidentified hydrocarbons in panels A, B, and C, respectively. Tentative identification of select peaks is (A1) butyric acid, (A2) pentanoic acid, (A3) hexanoic acid, (A4) heptanoic acid, (A5) octanoic acid, (B1) hexanal, (B2) tetradecane, (B3) octanal, (B4) hexanol, (B5) nonanal, (B6) heptanol, (B7) octanol. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

(approximately 3–9% moisture by mass; Wewala, 1993). By packing under a modified atmosphere with minimal residual headspace oxygen, commercial WMP has a shelf-life of 24 mo, if held below 25°C and 65% relative humidity (Fonterra, 2023), although skim milk powder, with comparatively little milkfat content, and stored in cans for emergency preparedness, is reported to have no deterioration of hedonic scores after 23 years of storage (Lloyd et al., 2004). It was only many years after Shackleton WMP was manufactured that it was understood that WMP with very low a_w (which the roller-drying process is prone to have) will tend to accelerate lipid oxidation (Labuza and Dugan, 1971; Wewala, 1993).

Aldehydes, alcohols, free fatty acids, and hydrocarbons may all arise as products of lipid oxidation. However, care is required with any speculation because such compounds may have had other sources. For instance, free fatty acids could also be due to lipolysis that requires the presence of water, and so may have developed in the raw milk after milking and before roller-drying. Likewise, hydrocarbons found in the Shackleton WMP could have been contaminants from combustion introduced at some early point in the history of the material.

Although comparing mass spectra from the qualitative chromatograms to library databases has limited validity for proving absence, it is still notable that Shackleton WMP did not appear to contain, with a high degree of certainty, any compounds that would seem wildly unusual, or out of place, for a powder with such a history. This reflects the prudent use of metal cans as the original packaging material (Figure 1A). The propensity of milk powder to adsorb volatile compounds would imply that if metallic (or equivalently impermeable) packaging had not been used, the volatile profile may well have reflected any other foods and supplies that the milk powder was stored with. Also, the relative absence of volatile compounds characteristic of Maillard reactions is consistent with storage at cold temperatures. Although Maillard reactions are substantially hindered by cold temperatures and low a_w, lipid oxidation is not hindered.

Bulk Composition

The bulk composition of the powders as received, on a moisture-free basis and on a fat- and moisture-free basis, is given in Table 2, along with the stated composition of the Shackleton WMP from the original label on the container (Figure 1C). As received, the Shackleton WMP had a higher moisture (7.75%) than stated on the container. As the Shackleton WMP container had been opened and resealed sometime early in its storage history, it is likely that some moisture ingress had oc-

Table 2. Bulk composition of whole milk powders (WMP)[1]

Component	As received				Moisture-free basis				Fat- and moisture-free basis			
	Sk WMP label	Sk WMP	WMP1	WMP2	Sk WMP label	Sk WMP	WMP1	WMP2	Sk WMP label	Sk WMP	WMP1	WMP2
Moisture (g/100 g)	4.9	7.75	3.36	3.06	—	—	—	—	—	—	—	—
Ash (g/100 g)	5.6	5.55	5.11	4.93	5.89	6.00	5.29	5.09	8.22	8.68	7.26	6.93
Protein[2] (g/100 g)	26.2	23.61	24.05	24.05	27.55	25.51	24.89	24.81	38.47	36.91	34.19	33.81
NPN (g/100 g)	—	0.24	0.23	0.20	—	0.25	0.23	0.21	—	0.37	0.32	0.28
Lactose (g/100 g)	36.3	36.50	46.90	43.90	38.17	39.44	48.53	45.29	53.30	57.08	66.68	61.71
Fat (g/100 g)	27.0	28.60	26.30	25.80	28.39	30.90	27.21	26.61	—	—	—	—
Free fat[3] (%)	—	27.50	1.30	1.20	—	29.71	1.34	1.24	—	—	—	—

[1]Sk WMP = Shackleton WMP. Sk WMP label: refers to the composition of the Shackleton whole milk powder given on the label of the container. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

[2]Total N × 6.38.

[3]% wt/wt of powder, corresponds to ~96% of the total fat for the Shackleton WMP and ~5% of the total fat for WMP1 and WMP2.

curred over the long storage period. The protein level was slightly lower, and the fat level was slightly higher than stated on the label, whereas the ash and lactose levels were very close to the stated compositions. The Shackleton WMP had more than double the moisture content, considerably less lactose, more fat and ash, and similar levels of protein (TN × 6.38) and NPN than WMP1 and WMP2.

The Shackleton WMP had a substantially higher free fat level (∼27.5% on a powder basis, 96% of the total fat) than WMP1 and WMP2 (about 1.3% of the powder, 5% of the total fat). This high free fat level is consistent with the CLSM images, which showed high levels of coalesced fat in the Shackleton WMP (Figure 2C). A high level of free fat is one of the known characteristics of roller-dried WMP when compared with spray-dried WMP (Koc et al., 2003; Courtois, 2013). This high free fat level, along with the caramel or cooked flavor, is one of the reasons roller-dried WMP is desired for confectionary products such as chocolate; however, the high free fat content also makes the roller-dried WMP more susceptible to lipid oxidation off-flavors than spray-dried WMP (Koc et al., 2003).

On a moisture-free basis, the Shackleton WMP had a composition that was reasonably close to that stated on the label with about 2% lower protein, 2.5% higher fat, 1% higher lactose, and similar ash/mineral matter. The Shackleton WMP had much lower lactose; and higher fat, protein, and ash than WMP1 and WMP2. When compared on a fat- and moisture-free basis, the Shackleton WMP had higher protein and ash and lower lactose than WMP1 and WMP2. The lower fat and protein levels in WMP1 and WMP2 compared with the Shackleton WMP will be a consequence of fat and protein standardization processes that are used in modern commercial milk powder manufacture to produce more consistent products (Walstra et al., 1999; Bylund, 2015).

Major Mineral Composition

The major mineral composition of the powders as received, on a moisture-free basis and on a fat- and moisture-free basis are shown in Table 3. On an as-received basis, the major mineral composition of the 3 powders were similar, with the exception of the sodium level,

Table 3. Major and trace mineral composition of whole milk powders (WMP)[1]

	As received			Moisture-free basis			Fat- and moisture-free basis		
Component	Sk WMP	WMP1	WMP2	Sk WMP	WMP1	WMP2	Sk WMP	WMP1	WMP2
Major mineral component (g/kg)									
Calcium	8.7	8.4	8.6	9.4	8.7	8.9	13.7	11.9	12.1
Citrate	12.8	11.5	10.6	13.9	11.9	10.9	20.1	16.3	14.9
Chloride	6.5	7.0	5.6	7.0	7.2	5.8	10.2	10.0	7.9
Potassium	10.7	10.6	10.4	11.6	11.0	10.7	16.7	15.1	14.6
Phosphate	12.8	13.1	12.8	13.9	13.6	13.2	20.0	18.6	18.0
Sodium	4.5	2.8	2.2	4.9	2.9	2.2	7.1	4.0	3.1
Magnesium	0.8	0.9	0.7	0.8	0.9	0.8	1.2	1.2	1.0
Phosphorus	6.9	6.4	7.0	7.5	6.6	7.2	10.9	9.1	9.9
Sulfur	2.1	2.3	2.2	2.3	2.4	2.3	3.3	3.3	3.1
Trace mineral component (mg/kg)									
Aluminum	1.6	<0.1	<0.1	1.7	—[2]	—	2.5	—	—
Antimony	<0.01	<0.01	<0.01	—	—	—	—	—	—
Arsenic	<0.02	<0.02	<0.02	—	—	—	—	—	—
Cadmium	0.0035	<0.002	<0.002	0.0038	—	—	0.0055	—	—
Chromium	<0.1	<0.1	<0.1	—	—	—	—	—	—
Copper	0.41	<0.26	0.29	0.44	—	0.30	0.64	—	0.41
Iodine	0.13	0.35	0.36	0.14	0.36	0.37	0.20	0.50	0.51
Iron	39.5	1.39	1.28	42.7	1.4	1.3	61.8	2.0	1.8
Lead	0.71	<0.01	<0.01	0.77	—	—	1.11	—	—
Mercury	<0.01	<0.01	<0.01	—	—	—	—	—	—
Manganese	0.37	0.17	0.19	0.40	0.18	0.20	0.58	0.24	0.27
Nickel	0.11	<0.1	<0.1	0.12	—	—	0.17	—	—
Nitrate	5.32	11.2	2.24	5.75	11.59	2.31	8.32	15.92	3.15
Nitrite	<0.3	<0.3	<0.3	—	—	—	—	—	—
Silver	<0.05	<0.05	<0.05	—	—	—	—	—	—
Tin	1.0	<0.03	<0.03	1.1	—	—	1.6	—	—
Zinc	27.3	23.4	25.2	29.5	24.2	26.0	42.7	33.3	35.4

[1]Sk WMP is Shackleton WMP. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

[2]A long dash indicates the component was below the detection limit.

which was markedly higher in the Shackleton WMP than WMP1 and WMP2. When viewed on a moisture-free basis, the differences between the Shackleton WMP and WMP1 and WMP2 were slightly greater due to the higher moisture content of the Shackleton WMP, and when viewed on a fat-and-moisture-free basis, the differences between the Shackleton WMP the WMP1 and WMP2 were again increased due to the higher fat content of the Shackleton WMP when compared with the 2 spray-dried milk powders.

On the fat-and-moisture-free basis, the sodium level in the Shackleton WMP was effectively double that of WMP1 and more than double WMP2. In addition, the calcium, citrate, phosphate/phosphorus, and potassium levels were somewhat higher in the Shackleton WMP; however, the differences were not that great and could be due to natural variations in the original milk used to make the powders, as well as standardization of protein with permeate used in the manufacture of WMP1 and WMP2. The sodium level in the Shackleton WMP is too great to be due to natural variations, and it is likely that some form of sodium salt has been added to the milk before powder manufacture. This was not due to the addition of common salt as the chloride levels were similar in the 3 powders. When looking at the original patent for roller-dried milk powder on which the manufacture of the Shackleton WMP was based, it states that, "To make a superior product, I prefer initially, ... to cut off the excessive acidity ... in case of whole milk to a shade on the alkaline side by the addition of lime or a soluble lime salt" (Just, 1902, p. 1). As the calcium level was only slightly higher in the Shackleton WMP, it is probable that sodium hydroxide was added to the whole milk to increase the pH to about neutral before roller-drying and milling to form the powder. This is consistent with the measured pH of the reconstituted milk (15% wt/wt), which was 6.95 for the Shackleton WMP, 6.62 for WMP1 and 6.64 for WMP2. Fresh cow milk has a pH value that ranges from 6.40 to 6.89 with an average of 6.63 (Tsioulpas et al., 2007).

Trace Mineral Composition

The trace mineral composition of the powders as received, on a moisture-free basis and on a fat-and-moisture-free basis are shown in Table 3. Many of the trace minerals were below the detection limit of the technique for all 3 powders and therefore will not be discussed further. Of the other trace minerals, there were some differences between the Shackleton WMP and WMP1 and WMP2. The level of lead was below detection levels in WMP1 and WMP2 but was at 0.71 mg/kg for the Shackleton WMP. If the milk powder was reconstituted to the original milk solids concentration,

this level of lead would be more than 3 times the Codex 0.02 mg/kg maximum level (Codex Alimentarius, 2019). The iron level in the Shackleton WMP was about $30\times$ higher than for the spray-dried milk powders at 39.5 mg/kg in the powder, as received, and 42.7 mg/kg on a moisture-free basis. Even concentrations of iron as low as 0.003 mg/kg can initiate oxidative off-flavors in liquid milk if they are introduced via contamination with processing water, whereas higher levels of iron (0.3–0.6 mg/kg) naturally present in liquid milk (equivalent to 2.4–5.8 mg/kg in WMP) are less prone to causing off-flavors due to the iron being bound by milk proteins (Mann et al., 2013) The tin level at 1.0 mg/kg for the powder, as received, was within the Codex 250 mg/kg maximum level for canned foods (Codex Alimentarius, 2019), but at 0.125 mg/kg (on a reconstituted liquid basis) was outside the expected range for milk. A survey that included 20 samples of liquid milk from Australia found a single sample with 0.05 mg/kg of tin with the remaining 19 samples all having <0.02 mg/kg (Callan et al., 2014).

Aluminum and nickel were below detection levels in WMP1 and WMP2 but were at elevated levels in the Shackleton WMP. Cadmium was also detected in the Shackleton WMP at very low levels but was not detected in either WMP1 or WMP2. Manganese was at about twice the level in the Shackleton WMP when compared with WMP1 and WMP2. Copper was not detected in WMP1, was at a moderate level in WMP2 and was about a third higher for the Shackleton WMP than for WMP2. Iodine was markedly lower in the Shackleton WMP at about a third the level of WMP1 and WMP2. The nitrate level of the Shackleton WMP was between those of WMP1 and WMP2.

It is unlikely that the high iron, lead, and tin levels were in the Shackleton WMP would have originated from environmental contamination of cow milk as, at the time that the milk powder was produced (~1907), New Zealand would have had too little opportunity to have accumulated pollution from anthropogenic activity. The high iron, lead, and tin levels are most likely a consequence of the manufacturing equipment used in the production of the milk powder, as well as the packaging into tin-plated cans that used lead-based solder seaming (Figure 1A). Although the powder was sampled from the center of the can, jostling of the milk powder particles within the can on the journey to Antarctica may have abraded tinplate and moved it from the inner surface toward the can's center. Stainless steel was not invented and used until the 1920s, therefore the manufacturing equipment would have used cast iron, and this may have contributed to the high iron levels in the finished product. In addition, it is possible that the water reticulation systems and other equipment of

that time may have used some lead and tin materials, and this may have resulted in the elevated lead and tin levels of the powder.

AA Analysis

The AA composition of the Shackleton WMP, WMP1, and WMP2 was determined and is presented on a protein basis (Figure 5A) to account for varia-

tions in the compositions of the powders. The only AA that was markedly different between the powders was lysine, which was about 20% lower in the Shackleton WMP (~1,550 mg/100 g) than WMP1 (1,925 mg/100 g) and WMP2 (1,950 mg/100 g). Mauron et al. (1955) considered lysine, methionine, and tryptophan to the most heat labile AA and examined their availability in fresh milk, spray-dried milk powder, and roller-dried milk powder. Only the lysine level was affected by the
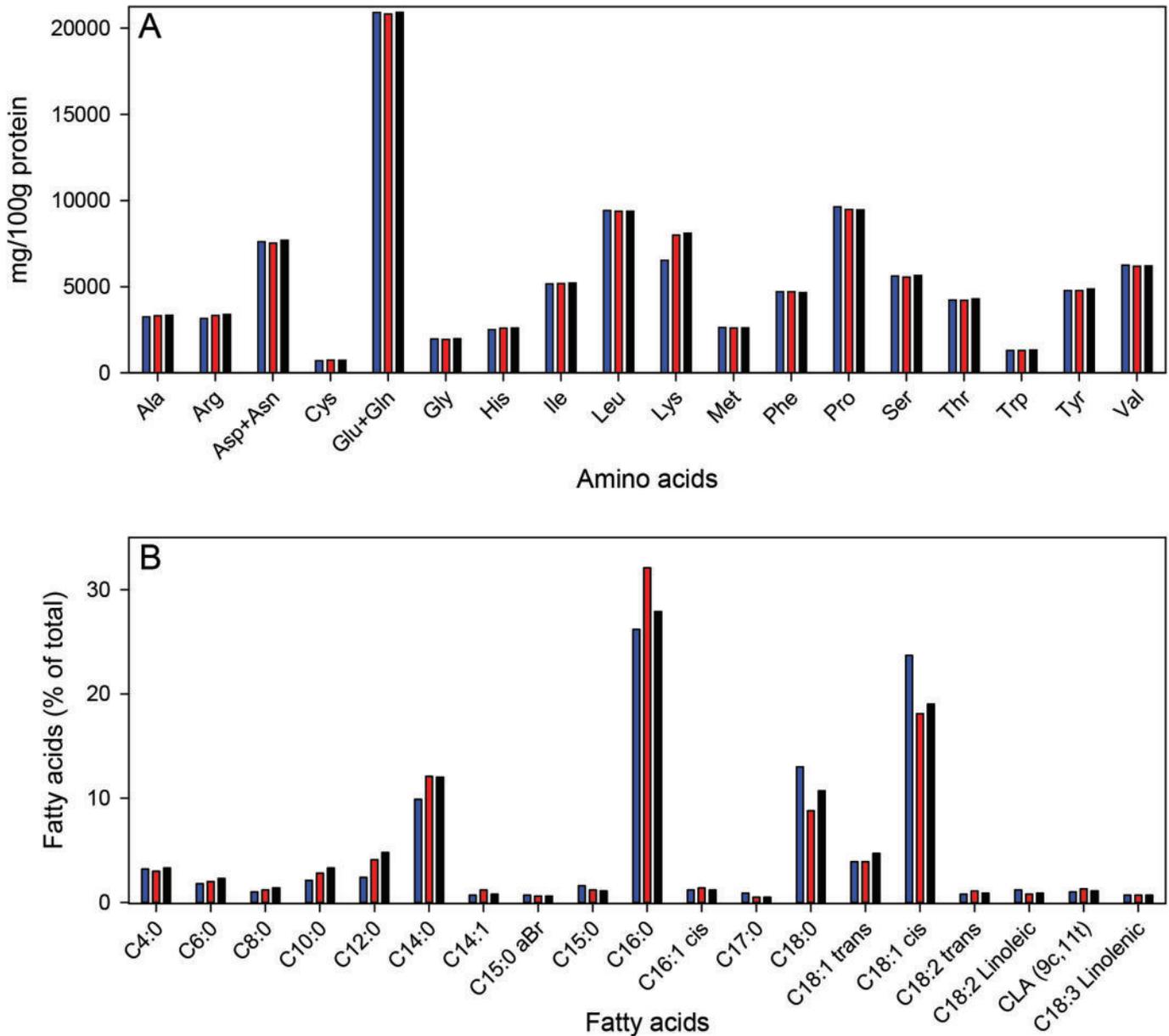
Figure 5. Amino acid composition (A) and fatty acid composition (B) of Shackleton whole milk powder (WMP; blue), WMP1 (red), and WMP2 (black). Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.
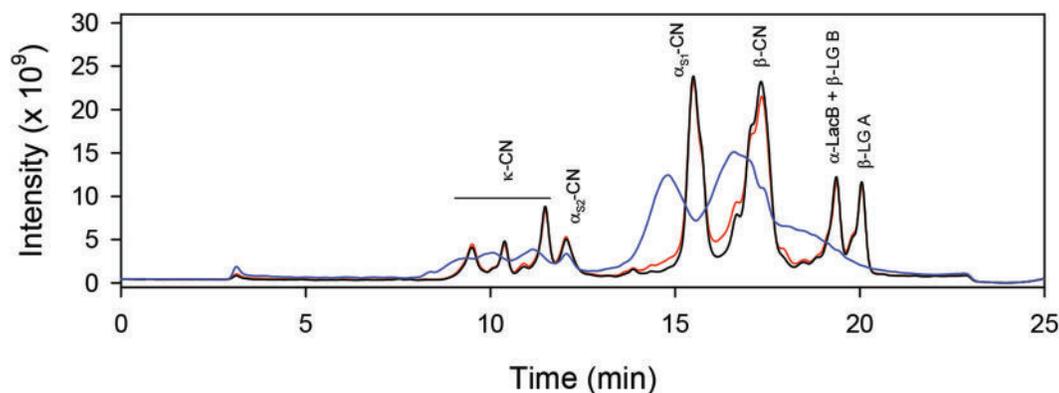
Figure 6. Total ion current of reverse-phase HPLC-MS trace of casein prepared from Shackleton whole milk powder (WMP; blue), WMP1 (red), and WMP2 (black). α-LacB refers to the B variant of α-LA; β-LGA and β-LGB refer to the A and B variants of β-LG, respectively. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

processing, being ~4% lower in the spray-dried milk compared with the fresh milk and between 15% and 25% lower in the roller-dried milk powder when compared with the fresh milk. The roller-dried milk powder had between 10% and 20% lower lysine levels than the spray-dried milk powders. In a subsequent study, Mauron and Mottu (1958) showed that the lysine availability, when compared with fresh milk, was reduced by between ~10% and 27% depending on the conditions used in manufacturing the roller-dried milk powder.

The milk powders used in the studies of Mauron et al. (1955) and Mauron and Mottu (1958) were prepared in the 1950s, almost 50 yr later than the Shackleton WMP and used vacuum evaporation to achieve a concentrate of ~40% TS before spray or roller drying. This is likely to have been a milder process to that used during the manufacture of the Shackleton WMP as vacuum evaporation, which was not introduced until 1913 (Bylund, 2015), allows for lower temperature conditions for drying of milk. Therefore, it seems that the lysine content of the Shackleton WMP compared with WMP1 and WMP2 is consistent with expected losses for a roller-dried powder manufactured in 1907 when compared with a spray-dried powder manufactured under modern processing conditions.

Fatty Acid Composition

Fatty acid analysis was performed on the milkfat from the WMP. Despite the vastly different feeding regimens and animal husbandry practices between when WMP1 and WMP2 were manufactured and when the Shackleton WMP was manufactured, the differences in the fatty acid compositions were relatively small. Figure 5B shows

the differences in the major fatty acids of the milkfat. The Shackleton WMP had higher levels of the C18:1 *cis* and C18:0 fatty acids, and lower levels of the C8:0, C10:0, C12:0, C14:0, and C14:1 fatty acids when compared with both spray-dried WMP. WMP1 had much higher levels of the C16:0 and lower levels of the C18:0 fatty acids than the Shackleton WMP and WMP2.

The information on seasonal and breed effects on the fatty acid composition of New Zealand milkfat has been studied and there are distinct seasonal effects on fatty acid composition (Hansen and Shorland, 1952; MacGibbon and McLennan, 1987; Sanjayaranj et al., 2022). The observation of high C18:1 and C18:2 and lower levels of the shorter-chain fatty acids suggest an early season (early spring) or late season (late autumn) milk was used for the powder manufacture, with possibly a Friesian dominant herd. Pasture-fed milk has higher CLA content than stall-fed cows (Robinson and MacGibbon, 2000; MacGibbon et al., 2001; Floris et al., 2006), and the significant and comparable levels of CLA in the 3 milk powders confirms that the powders were manufactured using milk from pasture-fed cows.

MS Analysis of Milk Proteins

It was not possible to characterize all the casein variants in the Shackleton WMP directly using the HPLC-MS because of poor ionization and resolution of the proteins (Figure 6). This poor resolution was likely to be due to the significant loss of charged AA, particularly free lysine (Figure 5A) because of extensive lactosylation of the proteins.

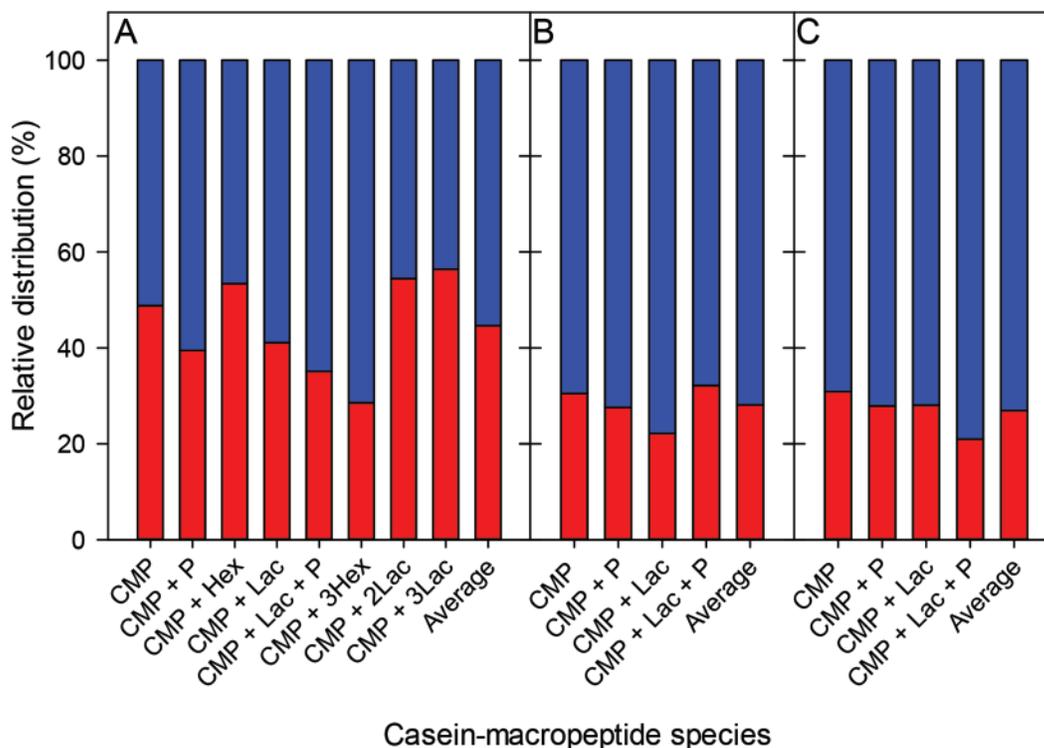The κ-CN variant distribution was determined indirectly using HPLC-MS by measuring the peak inten-

Figure 7. Relative distribution of casein-macropeptide (CMP) A (red bars) and CMP B (blue bars) variants and their various glycosylated products for Shackleton whole milk powder (WMP; A), WMP1 (B), and WMP2 (C). P = phosphate; Hex = hexose unit; Lac = lactose. Average = average distribution for all protein species observed. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

sities of their respective casein-macropeptide (**CMP**) products after rennet treatment of the milk. The rennet treated Shackleton WMP indicated that the κ-casein A and κ-casein B variants were present at a ratio of approximately 50:50. The rennet treated WMP1 and WMP2 had an observed ratio of the κ-CN A and κ-CN B variants of about 30:70 (Figure 7). Direct HPLC-MS analysis the intact κ-CN variants for WMP1 and WMP2 gave a ratio of approximately 35:65, which was close to that observed with the indirect method of measuring CMP A and CMP B (results not shown). This indicates that the indirect method gave a reasonably good indication of the κ-CN A to κ-CN B ratio. The Shackleton WMP contained more lactosylated CMP A and CMP B products compared with WMP1 and WMP2 (Figure 7). It is expected that majority of the lactosylation occurred during the high heat treatment of the roller-drying manufacturing process of the powder, rather than during long storage under the Antarctic conditions.

The difference in the κ-CN A and κ-CN B variant frequencies between the Shackleton WMP, WMP1, and WMP2 supports the suggestion that the Shackleton WMP is likely to be prepared from milk from a Friesian/Holstein dominant herd as Friesian/Holstein cows have a higher proportion of κ-CN A variant whereas Jersey cows have a lower proportion of the κ-CN A variant with a ratio closer to 20:80 A to B variant (Swaisgood, 2003; Farrell et al., 2004; Sanchez et al., 2020). The WMP1 and WMP2 may have been prepared from Jersey dominant herds; however, selective breeding for certain traits may have shifted the frequency to relatively more κ-CN B variant regardless of the breed.

Although the ionization of the β-CN variants was poor (Figure 6), there was still sufficient signal to allow the mass spectrum to be deconvoluted to determine the relative distribution of the major β-CN variants. The A1, A2, and B variants of β-CN were present at 31%, 59%, and 10%, respectively, which was similar with that observed for WMP1 and WMP2, which had distributions of 29%, 60%, and 11%, respectively (Figure 8A). Lactosylation of up to 2 lactose units were detected in the β-CN variants. However, when the WMP samples were digested with plasmin, it was possible to identify γ2-CN, γ3-CN, and proteose-peptose-5 peptides generated from all the β-CN variants. For the Shackleton
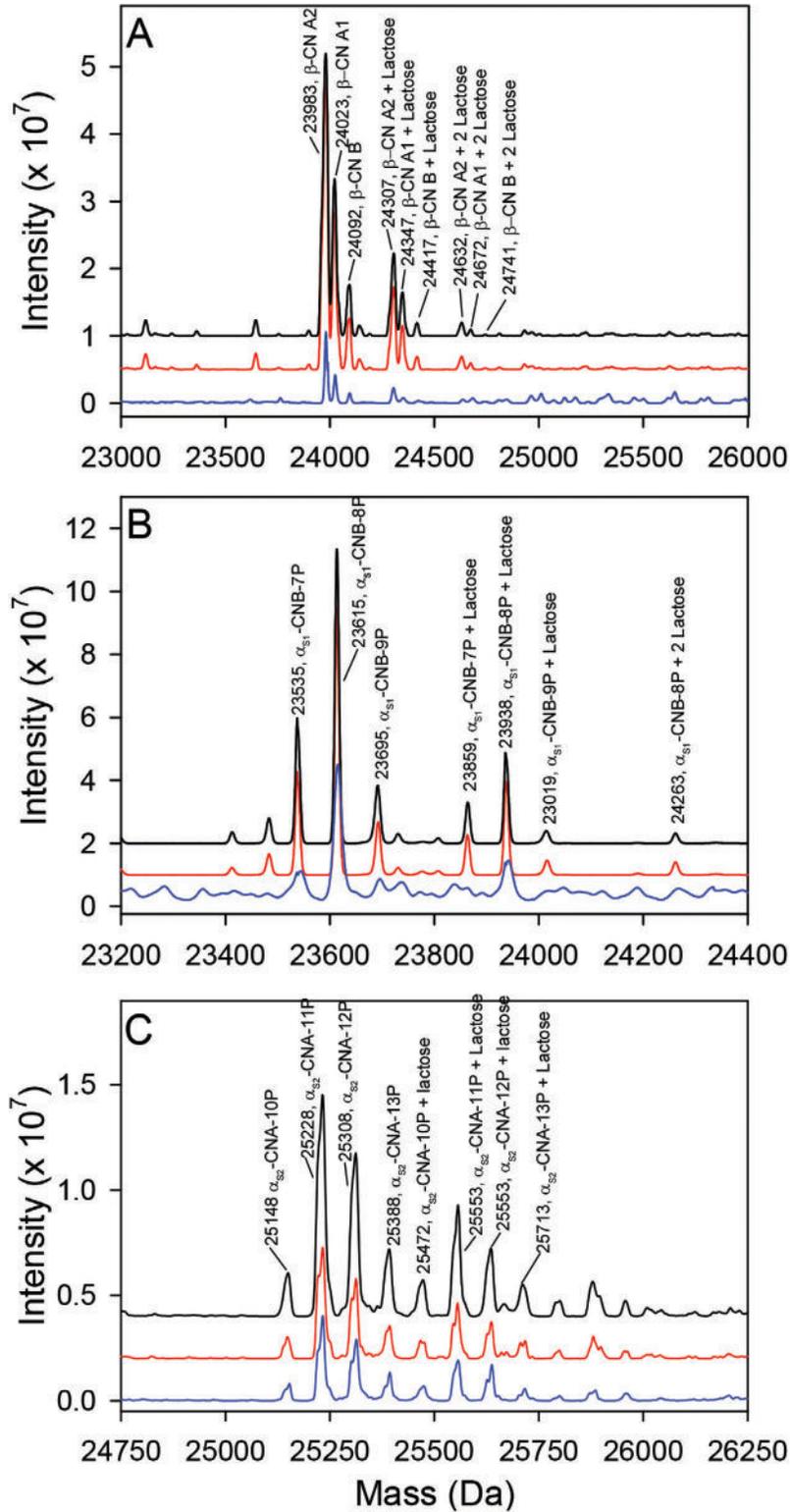
Figure 8. The deconvoluted mass spectrum for β-CN (A), α_{S1}-CN (B), and α_{S2}-CN (C) for Shackleton whole milk powder (WMP; blue), WMP1 (red), and WMP2 (black). The mass spectra show the distributions of genetic variants (observed for β-CN only), the phosphorylated species (P), and lactosylated species. Only identified protein species have been annotated. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

WMP sample, some of these peptides showed extensive lactosylation with up to 4 bound lactose molecules detected (results not shown).

α_{S1}-Casein B had poor ionization in the Shackleton WMP, compared with WMP1 and WMP2. In all milk powders the α_{S1}-CN B-8P and B-9P were detected along with the single lactosylated products of α_{S1}-CN B-8P and B-9P and the double lactosylated products of α_{S1}-CN B-8P (Figure 8B). In all 3 WMP samples, the α_{S1}-CN B-8P was the major casein detected. This is consistent with expectations as the predominant α_{S1}-CN component in the milk of western cattle is α_{S1}-CN B-8P with low levels of α_{S1}-CN B-9P (Farrell et al., 2004). Interestingly, the literature indicates that α_{S1}-CN B-7P is not a significant component of casein (Farrell et al., 2004); however, all 3 milk powders had a peak with an average mass consistent with α_{S1}-CN B-7P, and this peak was at a higher level than α_{S1}-CN B-9P (Figure 8B), which is not consistent with literature reports. The mass associated with this peak does not correspond to the C variant or the very rare A variant of α_{S1}-CN. It is possible that New Zealand has a higher content of α_{S1}-CN B-7P than observed elsewhere, and that this situation has existed in New Zealand since the time of manufacture of the Shackleton WMP. Comprehensive identification of this peak would need more extensive evaluation, which was beyond the scope of this comparative study.

The major α_{S2}-CN variant determined in the 3 WMP was the A variant, which is consistent with expectations (Farrell et al., 2004). The Shackleton WMP had a similar post-translational phosphorylation distribution as those for WMP1 and WMP2, with the major phosphorylation component being α_{S2}-CN A-11P (~30%), with lower levels of the α_{S2}-CN A-10P, A-12P, and A-13P components (Figure 8C). This is consistent with literature, which reports the A variant as the predominant variant and the incorporation of 10 to 13 phosphate groups with the α_{S2}-CN A-11P being the predominant form (Swaisgood, 1992; Farrell et al., 2004). Lactosylated species of all α_{S2}-CN components were also detected (Figure 8C).

The lack of detection of more extensive lactosylation products for the casein in the Shackleton WMP when compared with WMP1 and WMP2 is likely to be due to poor ionization of these lactosylated proteins in the mass spectrometer and thus these species are underrepresented. However, as lactosylation is expected to be similar among the different casein components, the relative proportions of the genetic variants detected is expected to be consistent with that actually present in the powder and thus representative of the protein distribution in the milk samples used to manufacture the Shackleton WMP.

As with the casein proteins, ionization of the major whey proteins (α-LA and β-LG) from the Shackleton WMP was poor when compared with WMP1 and WMP2 (Figure 9). This was also likely due to the extensive lactosylation, which is usually evidenced by the broad peaks and poor resolution for these proteins. Despite this, it was still possible to pick up details of the whey protein variants in the Shackleton WMP, although it was at a lower intensity when compared with WMP1 and WMP2 (Figure 9).

For α-LA, the B variant was detected across all 3 WMP samples, with minor levels of the single lactosylation protein species (Figure 9A). This is as expected as the B variant is the dominant variant in *Bos taurus* cattle (Swaisgood, 1982; Farrell et al., 2004). For the β-LG, both the A and B variants were detected across the 3 WMP samples (Figure 9B). Their relative distribution was about 60:40 for the A and B variants in WMP1 and WMP2. This distribution was also reflected in their single and double lactosylation species, although triple lactosylation was only observed for β-LG A at very low levels. In contrast, for the Shackleton WMP, the observed β-LG A-to-B ratio was approximately 15:85. Unlike WMP1 and WMP2, the Shackleton WMP had more extensive lactosylation of β-LG, and the lactosylation was associated more with the A variant than the B variant (Figure 9B). The lower ratio of β-LG A to B variants may be due to the β-LG A being selectively lactosylated at a higher level than the B variant, although there is the possibility that the milk used in the manufacture of the Shackleton WMP came from cows that had a higher frequency of β-LG B variants. Previous studies have not shown any preferential lactosylation of the A variant of β-LG over the B variant (Leonil et al., 1997; Morgan et al., 1997; Anema et al., 2006), although none of those studies were on roller-dried milk samples.

MS Analysis of Phospholipids

The total phospholipid measured in the Shackleton WMP was 115 mg/100 g, which was markedly lower than WMP1 (315 mg/100 g) or WMP2 (213 mg/100 g). The results for the individual phospholipid classes (Figure 10A) showed that 4 glyceryl phospholipid classes (PE, PC, PS, and PI) were lower in the Shackleton WMP, with PE registering the largest difference, being more than 80% lower than in WMP1 or WMP2. There was no evidence that the reduction of PE and PC in the Shackleton WMP was due to hydrolysis as their respective hydrolysis products, LysoPE (0.1 mg/100 g) and LysoPC (1.4 mg/100 g), were not significantly elevated. However, the lactosylated PE, which is a reaction product of the Amadori rearrangement reaction (Amadori,
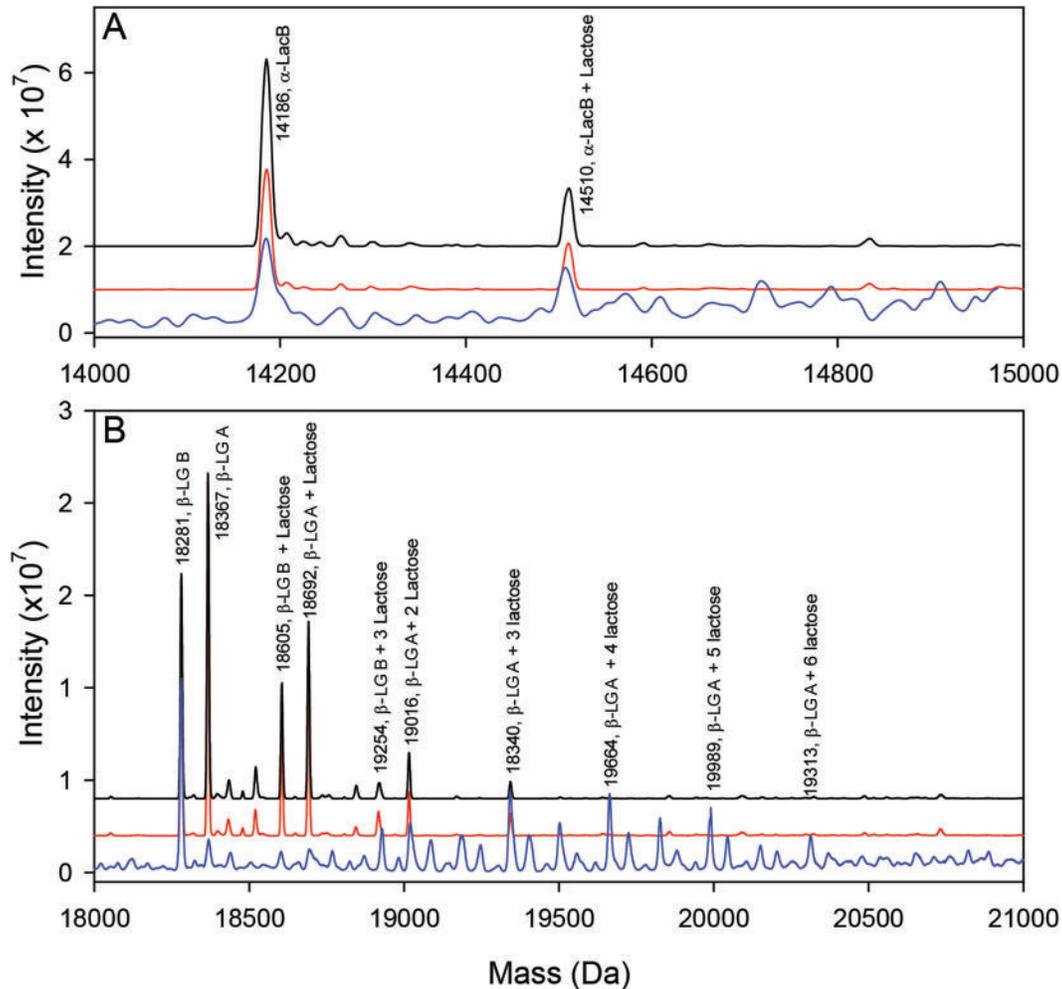
Figure 9. The distribution of α-LA (A) and β-LG (B) variants, and their respective lactosylation species in Shackleton whole milk powder (WMP; blue), WMP1 (red), and WMP2 (black). Only identified protein species have been annotated. α-LacB refers to the B variant of α-LA; β-LGA and β-LGB refer to the A and B variants of β-LG, respectively. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

1925) of lactose and the amine group of PE, was elevated and estimated at 26% of the total phospholipids in the Shackleton WMP compared with WMP1 and WMP2 at 7% and 5% of total phospholipids, respectively. The lactosylated PE was only estimated in this study using a single molecular species standard; however, this lactosylation of the PE in the Shackleton WMP may partially account for the lower level of PE detected in this powder. Sphingomyelin (**SM**), typically classed as a phospholipid, also belongs to the sphingolipid family. Unlike the 4 glyceryl phospholipids, the SM levels measured in the Shackleton WMP were within the typical ranges and similar with those observed for WMP1 and WMP2 (Figure 10A).

Bovine milk phospholipid concentration is generally influenced by stage of lactation and season. In New Zealand, the milk phospholipid levels are generally lowest in spring (calving) peaking around mid to late summer and decreasing in autumn. However, the relative phospholipid class distribution remains relatively constant, as observed for WMP1 and WMP2 (Figure 10). The large decrease in the glyceryl phospholipid observed in the Shackleton WMP has altered the relative distribution of the phospholipid classes compared with the WMP1 and WMP2 (Figure 10B).

Hexosylceramides and LacCer are glycosphingolipids that, like SM, also belong to the sphingolipid family. The level of HexCer and LacCer in the Shackleton
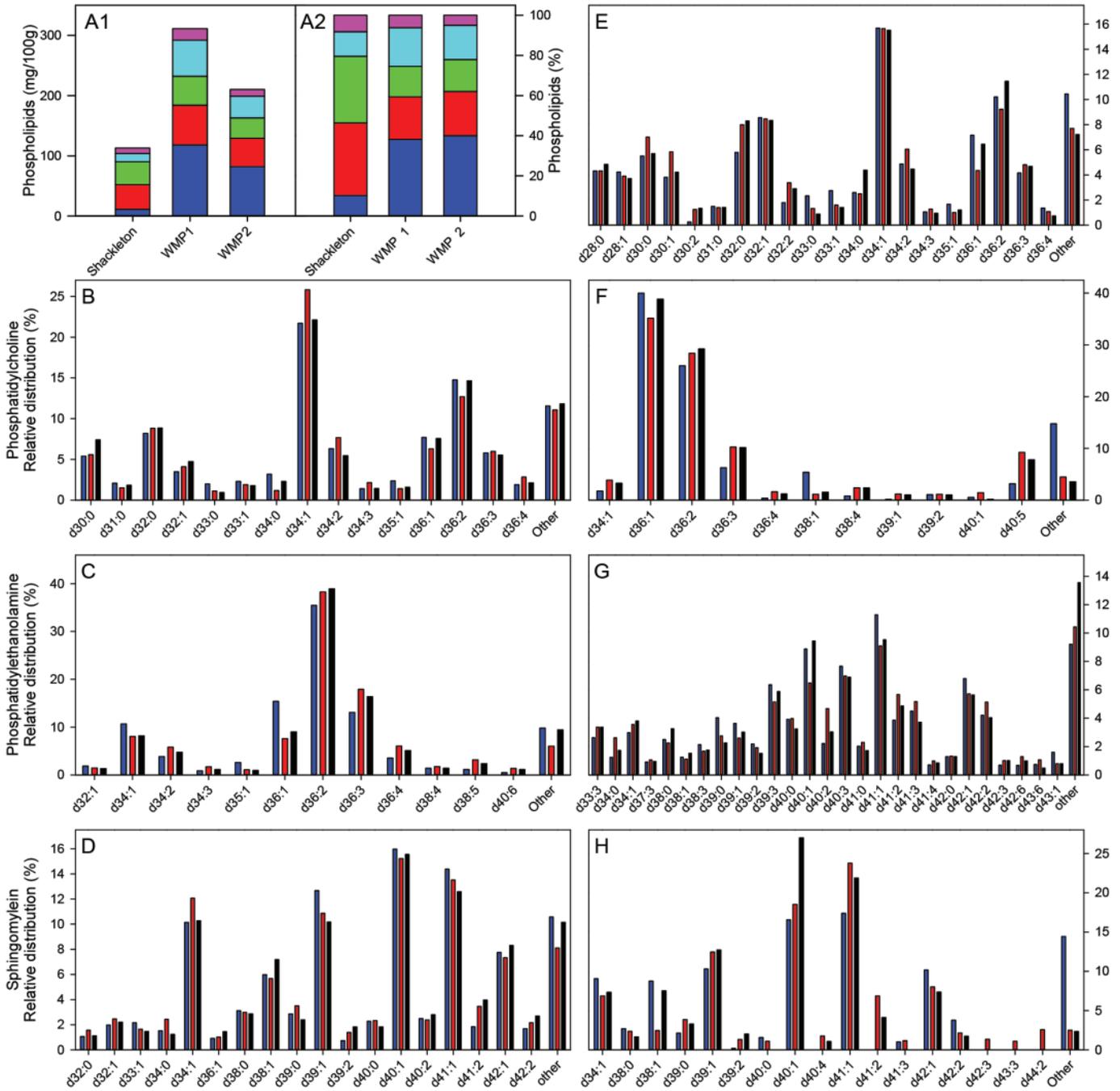
Figure 10. The phospholipid concentration (A1) and relative phospholipid class distribution (A2) for phosphatidylethanolamine (blue), phosphatidylcholine (red), sphingomyelin (pink), phosphatidylserine (green), and phosphatidylinositol (cyan). Molecular species distributions of phosphatidylcholine (B), phosphatidylethanolamine (C), sphingomyelin (D), phosphatidylinositol (E), phosphatidylserine (F), hexosylceramide (G), and lactosylceramide (H) in Shackleton whole milk powder (WMP; blue bars), WMP1 (red bars), and WMP2 (black bars). For panels B to H, the values on the x-axis are the D-values. The D-values for sphingomyelin (D) are the sum of the carbon atoms and unsaturated bonds associated with the sphingosine base and acyl fatty acids, whereas for the glycerol phospholipids (B, C, E–H) the values are the sum of the carbon atoms and unsaturated bonds of the acyl fatty acids attached to the glycerol backbone. Shackleton WMP is a roller-dried whole milk powder manufactured in 1907 in Bunnythorpe, New Zealand. It was recovered from Shackleton's hut in Antarctica. WMP1 and WMP2 are modern-day commercial spray-dried whole milk powders, standardized for protein content (by permeate addition) and fat content. WMP1 was manufactured at Fonterra's Darfield factory and WMP2 was manufactured at Fonterra's Pahiatua factory, both in New Zealand.

WMP was not too dissimilar from the WMP1 and WMP2 (results not shown). These results suggest that sphingolipids may be more stable during prolonged storage than the glyceryl phospholipids. The larger losses of glyceryl phospholipids compared with the sphingolipids may be due to their molecular species. Glyceryl phospholipids tend to contain more PUFA (D-values less than 40 with MUFA, di-UFA, and tri-UFA; for glycerol phospholipids the D-values are the sum of the carbon atoms and unsaturated bonds of the acyl fatty acids attached to the glycerol backbone, whereas for sphingolipids it is the sum of the carbon atoms and unsaturated bonds associated with the sphingosine base and acyl fatty acids), making it more susceptible to oxidation. In contrast, SM and LacCer have a D-value above 39 with predominantly MUFA. The exception is HexCer which contain reasonable amounts of MUFA, di-UFA, and tri-UFA.

Despite the difference observed in the glyceryl phospholipids, there were not a lot of differences in their relative molecular species distributions (Figure 10B, 10C, 10E, and 10F). Similarly, glycosphingolipids molecular species between the Shackleton WMP, WMP1, and WMP2 were also not too different (Figure 10D, 10G, 10H). This suggests there were no selective losses or changes of the phospholipids at their molecular species level in the Shackleton WMP during manufacture and storage over more than 100 yr under Antarctic conditions.

CONCLUSIONS

Despite more than a century between the manufacture of the Shackleton WMP and WMP1 and WMP2, the composition of bulk components and detailed protein, fat, and minor components has not changed drastically in the intervening years. The differences observed in bulk composition would be largely due to the lack of standardizing procedures used in the manufacture of the Shackleton WMP, whereas both fat and protein standardizing were used in WMP1 and WMP2. The fatty acid composition, phospholipid composition, and protein composition including casein and whey protein genetic variations were, in general, remarkably similar between the Shackleton WMP and WMP1 and WMP2.

The Shackleton WMP had high free fat levels, low lysine levels, and evidence of considerable Maillard/lactosylation reactions compared with WMP1 and WMP2. In addition, the microstructural analysis revealed major differences in the morphologies of the powders, with the Shackleton WMP being flakes with high levels of coalesced fat, whereas WMP1 and WMP2 were agglomerated spheres and had predominantly intact fat globules. These differences are attributable to the manufacturing processes used with the roller-drying process used in the Shackleton WMP being formed from milled films of dried milk, which produces high levels of coalesced free fat and increased Maillard reactions products compared with the spray-drying process used in WMP1 and WMP2.

The major mineral components were similar between the milk powders with the exception of high sodium levels in the Shackleton WMP indicative of pH adjustment of the milk with a sodium hydroxide solution before drying. The high levels of lead and tin in the Shackleton WMP are probably contaminants from the tin-plated can with soldered seams; and the high content of iron, and other trace minerals in the Shackleton WMP are probably contaminants arising from the manufacturing equipment and water supply of the day, which have been essentially eliminated from modern milk powders through the use of stainless steel and quality water services.

One of the major differences between the milk powders was the qualitative volatile profile, which revealed components in the Shackleton WMP that would be consistent with substantial oxidative off-flavors, whereas these components were essentially absent in WMP1 and WMP2. It is probable that, despite favorable storage conditions, there was continuing lipid oxidation leading to the increased volatile components. However, it is also possible that collection and storage of the raw milk before drying were less than ideal, and that some deleterious reactions may have occurred before the manufacture of the milk powder.

Despite the remarkably similar composition of the milk powders, the spray-dried WMP1 and WMP2 were substantially superior to the roller-dried Shackleton WMP in terms of powder attributes, especially solubility and appearance. Therefore, unlike the whisky discovered in Shackleton's hut, it is highly unlikely that the milk powder manufactured in 1907 will be recreated to produce a "Shackleton Whole Milk Powder." However, in the era of polar explorers, where sufficient food supplies to last years had to be carried in the limited space available on the ships of the period, dried foods rich in protein and energy and easily prepared into consumable form, such as the roller-dried WMP, would have been a highly valuable commodity. It is appropriate to leave the last words to Ernest Shackleton himself: "Some of us preferred a cup of hot fresh milk, which was easily made from the excellent dried milk of which we had a large quantity" (Shackleton, 1909, p. 208). One can imagine that the hot milk may have been made with powder from the same tin-plated containers that we have subsampled and analyzed over a century later.

ACKNOWLEDGMENTS

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