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The Impact of the Skim Milk Powder Manufacturing Process on the Flavor of Model White Chocolate

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ABSTRACT

Milk powder is an important ingredient in the confectionary industry but its variable nature has consequences for the quality of the final confectionary product. This paper demonstrates that skim milk powders (SMP) produced using different (but typical) manufacturing processes, when used as ingredients in the manufacture of model white chocolates, had a significant impact on the sensory and volatile profiles of the chocolate. SMP was produced from raw bovine milk using either low or high heat treatment, and a model white chocolate was prepared from each SMP. A directional discrimination test with naïve panellists showed that the chocolate prepared from the high heat SMP had more caramel/fudge character ($p<0.0001$), and sensory profiling with an expert panel showed an increase in both fudge ($p<0.05$) and condensed milk ($p<0.05$) flavor. GC-MS and GC-Olfactometry of both the SMPs and the model chocolates showed a concomitant increase in Maillard-derived volatiles which are likely to account for this change in flavor.

Keywords: milk processing, skim milk powder, white chocolate aroma, GC-MS, GC-O,

INTRODUCTION

Milk powder is an important confectionery ingredient, used in products such as toffees, caramels and fudges, as well as in white and milk chocolate. However, the role of milk powder in flavor formation during confectionery manufacture remains poorly understood. The aroma profile of milk chocolate has been thoroughly investigated[1,2] and since many of the desirable flavor characteristics are derived from cocoa solids, comparisons have been made with the aroma profiles of other cocoa-containing products such as dark chocolate,[2-4] cocoa powder[5], roasted cocoa[6,7] and cocoa liquor.[2] The aroma profile of white chocolate has not previously been investigated and it provides an ideal base in which to investigate the aroma compounds present in chocolate which are derived from the milk powder, excluding those which are derived from the cocoa solids.

Milk powder is used in confectionery production where a low moisture environment is required. For example, the moisture content of chocolate must remain below 1.5% to prevent interactions between water and sugar which increase the viscosity of the product.[8] The quality of milk powder available, and the processing conditions applied during production, are highly variable and heat treatment in particular can vary from pasteurization alone (15 s at 72 °C) to more severe processing, depending on the final properties required. For example, high heat milk powder can be produced by applying a heat treatment of 120-135 °C for 2-3 min.[9]

Turner et al.[10] studied the effect of heating on the aroma of SMP, showing that a number of Maillard-derived compounds, such as 2,3-butanedione and 2-furfural, were produced at 90 °C. Karagül-Yüceer et al.[11] used aroma extract dilution analysis (AEDA) to compare the aroma of commercial SMP samples prepared with different heat treatments (low, medium and high). They concluded that volatile compounds derived from thermal reactions were fundamental to SMP aroma, with compounds such as 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) and free fatty acids perceived to have higher flavor dilution factors in high-heat SMP. Similarly, Kobayashi et al.[12] used AEDA and sensory evaluation to compare the characteristic odorants of high-heat SMP and UHT milk. Whereas UHT milk was scored more highly for milky attributes, resulting from higher levels of lactones, brothy notes were given higher scores in high heat SMP, attributed to the presence of sulfur compounds. In both studies, the heating conditions used to produce the different powders were not specified, as the powders were obtained from commercial sources.

Pistokoulou et al.[13] used solvent assisted flavor evaporation (SAFE) and AEDA to identify aroma compounds responsible for a cooked-milk note present in milk after mild heat treatment more typical of domestic processing. Fatty acids were present in all samples and showed some of the highest odor activity values. Shiratsuchi et al.[14] also found these compounds to be the major contributors to the flavor of spray-dried SMP, and also identified lactones in skim milk powder, whereas Pistokoulou et al. identified lactones in whole milk samples only. Thermally-derived compounds are considered as off-flavors in milk powder consumed as a final product (as a milk substitute), but compounds such as 2,3-butanedione (creamy/buttery odor) have the potential to contribute positively to the flavor profile of confectionery.[1]

The aim of this work was to determine whether SMP manufactured under different conditions, when used as an ingredient in the manufacture of a model white chocolate, had a significant impact on the sensory and volatile profile of the final product. The impact of the standard thermal processes used during the manufacture of milk powder has not been previously investigated. Two batches of SMP were prepared from the same batch of raw milk and the process carefully controlled to ensure that the only difference between the batches was in the heating step traditionally applied prior to spray drying. A model white chocolate was selected for this study because of its relative simplicity compared to milk chocolate, where the incorporation of cocoa solids influences both the chemistry and the sensory properties of the product. Two batches of white chocolate were prepared and compared using discrimination tests, sensory profiling, GC-Olfactometry and GC-MS.

MATERIALS AND METHODS

Chemicals. Aroma chemical were obtained from the following suppliers: 2,3-diethyl-5-methylpyrazine and 2-furfural from Acros (Fisher Scientific, Loughborough, UK); 2-acetyl-1-pyrroline and maltol (methyl d3) from AromaLab (Planegg, Germany); 1-octen-3-one from Danisco (Kettering, UK), γ-decalactone, δ-decalactone, δ-dodecalactone, benzaldehyde, butanoic acid, hexanoic acid and 4-hydroxy-5-methyl-3(2H)-furanone (norfuraneol) from Givaudan (Milton Keynes, UK); (*E,E*)-2,4-decadienal from Lancaster Synthesis (Heysham, UK); 2-furanmethanol from Oxford Organics (Hartlepool, UK); (*E,E*)-2,4-nonadienal, 2,3,5-trimethylpyrazine, 2,3-butanedione, 2- methylbutanoic acid, 3-methylbutanoic acid, 2-methyl-3-(methyldithio)furan, acetic acid, decanal, dimethyl trisulfide, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), heptanal, hexanal, 3-hydroxy-2-methyl-4H-pyran-4-one (maltol), 3-methylsulfanylpropanal (methional), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), undecanal, (*Z*)-4-heptenal, 2H-furan-5-one, 2-methylpropanoic acid, 5-(hydroxymethyl)furfural, nonanoic acid, nonanal, (*E*)-2-nonenal, (*E*)-2-octenal, (*E*)-2-undecenal, (*E,E*)-2,4-octadienal, decanoic acid, γ-dodecalactone, 2-nonanone, dimethyl sulfone, tetramethylpyrazine, 2-isobutyl-3-methoxypyrazine and 2-methyl-3-heptanone from Sigma Aldrich Ltd. (Gillingham, UK); 1-octen-3-ol, γ-octalactone, δ-octalactone, octanoic acid, pentanoic acid and propanoic acid from Synergy (High Wycombe, UK). Repurified diethyl ether (DEE) was prepared by distilling 99% purity anhydrous DEE (Sigma) through a

Vigreux column (30 cm, 4 mm glass beads, distilled at 40 °C). HPLC-grade water was obtained from Fisher Scientific (Loughborough, UK). Alkane standard C_5-C_{30} (100 µg/µL in diethyl ether) was also obtained from Sigma-Aldrich Co. Ltd.

Production of SMP. The process is summarised in Figure 1. Raw bovine milk (113 kg) supplied by The University of Reading CEDAR Dairy Farm (CEDAR, Reading RG2 9HX, UK) was pasteurized at 72 °C for 15 s and separated using a disc bowl centrifuge to produce skim milk.

Concentration of skim milk. Skim milk was concentrated to ~20 % (w/w) solids using a rising film evaporator (T = 54 – 55 °C). The concentrated milk was divided into two batches of equal size. One batch was subjected to heat treatment (see below) to produce a high heat skim milk powder while the other batch was used directly (no additional heat treatment) to produce a low heat skim milk powder.

Heat treatment. One batch of concentrated milk was sealed into metal cans (3 L per can) and heated in a vertical retort at 125 °C for 5 min. It took approximately 10 min to reach a temperature of 125 °C inside the retort, from which time the 5 min heating period was measured. After heating it took approximately 5 min to reduce the pressure and remove the cans from the retort, after which the sealed cans were placed in cold water. These conditions were selected based on previous literature.[9]

Spray drying. Both batches of concentrated milk (one with a heat treatment, one without a heat treatment) were spray-dried to ~5% moisture using a NIRO spray dryer (Copenhagen, Denmark) with an A/S NIRO atomizer. The inlet air temperature was fixed at 200 °C and the feed flow rate was adjusted to give an outlet air temperature of 80 – 90 °C. The wet bulb temperature during spray drying was 45 – 50 °C. These two batches of milk powder (low heat skim milk powder (LHSMP) and a high heat skim milk powder (HHSMP)) were used to prepare two corresponding batches of model white chocolate, LHCHOC and HHCHOC respectively.

Measurement of milk components. The protein, fat, lactose and total solids content were measured throughout processing using a Lactoscope (Quadrachem Laboratories Ltd. London, UK), and the results are shown in Table 1.

Production of White Chocolate. The milk powders, prepared as described above, were used to manufacture two different model white chocolates. Sugar (4.57 kg), deodorized cocoa butter (1.89 kg), pasteurized milk fat (0.75 kg) and skim milk powder (LHSMP or HHSMP, 2.79 kg) were mixed thoroughly using a mixer with a beater attachment (Model K175, Crypto Peerless Ltd., Birmingham, UK) and refined to a particle size of 25 – 35 μm using a 3-roll refiner (Model SDX 600, Buehler, Uzwil, Switzerland) in two passes. The majority of the refined mix (7.47 kg) was transferred to a 10 kg Conche (Model IMC-E10, Lipp, Mannheim, Germany) and cocoa butter (0.25 kg) was added to make the mixture into a paste. The white chocolate was conched for 4 h at 50 °C, adding lecithin (0.032 kg) and the remaining cocoa butter (0.26 kg) for the final 30 min. After conching, the molten model chocolate was sieved and tempered by heating to 45 °C, cooling to 26.5 °C and finally bringing the temperature up to 27.5 °C. The tempered chocolate was moulded into 100 g bars and allowed to cool completely. The bars were sealed in metallic foil bags and stored at room temperature until use.

Discrimination testing. A panel of naïve volunteers (n = 50) was recruited from university staff and students who were willing to evaluate white chocolate, had no relevant food allergies and who provided written consent. Testing took place in individual sensory booths, at a controlled room temperature of 23±0.5 °C, and data were collected using Compusense 5 software (Compusense Inc., Guelph, Ontario, Canada). Assessors were provided with a glass of warm water for palate cleansing between samples. Samples were labelled with random 3-digit codes and presented in a balanced order under red lights, to minimize any color difference between products. Two forced choice discrimination tests were performed; a non-directional triangle test and, separately, a directional two-alternative forced choice (2-AFC) test. During the non-directional triangle test, assessors were presented with three samples of white chocolate. Two of the samples were identical and the other one different. Assessors were asked to taste the samples and state which product they believed to be the odd one out. During the directional 2-AFC test, assessors were presented with one sample of white chocolate prepared from low heat milk powder (LHCHOC) and one sample of white chocolate prepared from high heat milk powder (HHCHOC). Assessors were asked to taste both samples and state which sample they perceived to have "more caramel flavor".

Sensory profiling. A panel of nine trained assessors, each with a minimum of six months experience, was used to develop a quantitative descriptive analysis (QDA) sensory profile describing the sensory characteristics of different white chocolate samples. A sample of each model white chocolate (LHCHOC and HHCHOC) was presented to each assessor labelled with a random symbol. To develop the vocabulary for the sensory profiling, assessors were asked to smell, taste and finally swallow the samples to produce a list of descriptive terms for the appearance, odor, taste, flavor and mouthfeel of the samples and also for the attributes which lingered in the mouth after 60 s. Following this initial collection of terms, reference materials (Table S1) were provided. These terms were discussed by the panel of assessors as a group, assisted by a panel leader, to agree a final profile consisting of 2 appearance terms, 7 odor terms, 9 taste/flavor terms, 11 mouthfeel terms and 5 after-effect terms. A full list of terms is given in Table S2. The quantitative sensory assessment took place in individual sensory booths under red light and at room temperature controlled to 23±0.5 °C. Assessors were provided with a glass of warm water and unsalted crackers for palate cleansing between samples. Samples were presented to the assessors in a balanced order and assessors were asked to smell, taste and swallow the samples and score them on appearance, odor, taste, flavor and mouthfeel attributes. There was a 60 s pause after scoring the mouthfeel attributes, after which the assessors scored the samples for after-effects. The intensity of each attribute was recorded on an unstructured line scale (scaled 0-100) and all data were collected using Compusense 5 software (Compusense Inc., Guelph, Ontario, Canada). A duplicate assessment was carried out in a separate session.

Preparation of Extracts for GC-MS, GC-O and AEDA. Milk powders (15 g) were reconstituted using 100 mL HPLC-grade water, and 30 µL 2-methyl-3-heptanone (6.18 µg/25 mL) in methanol was added as an internal standard, before samples were stirred for 30 min. Reconstituted milk samples were added to 250 mL wide mouth Teflon screw cap bottles with 9 g solid NaCl to break the emulsion during extraction. Repurified DEE (99% purity, 100 mL) was used to extract the volatiles. Bottles were shaken every 10 min for 60 min, and then centrifuged at 4 °C for 20 min at 2990 × *g*. After centrifugation, the organic supernatant was carefully removed. The solvent-assisted flavor evaporation (SAFE) technique described by Engel *et al.*[15] was used to separate the volatile fraction of the milk (distillate) from any non-volatile residue

White chocolate (200 g) was cut into pieces, frozen in liquid nitrogen, and ground to a fine powder using a coffee grinder (DeLonghi KG49, Hampshire, UK). The powder was combined with DEE (800 mL), 2-methyl-3-heptanone (30 µL, 6.18 µg/25 mL methanol) was added as an internal standard, and maltol-(*methyl*-d_3) (17 µL, 2g/L in ethyl acetate) was added in order to quantify the maltol using stable isotope dilution analysis. The mixture was stirred well and left overnight. After filtering (Whatman No. 1 filter paper) to remove any solid material, the extract was distilled by SAFE, using the same method as for milk powder.

Extracts were dried over anhydrous sodium sulfate and then concentrated to 500 µL using a Vigreux column (50 cm × 1 cm internal diameter; VMR International, UK). The extracts were divided into two equal parts, and concentrated further to 100 µL. Each extract was prepared in triplicate, to give twelve samples in total, and stored at -80 °C before analysis.

GC-Olfactometry and Aroma Extract Dilution Analysis (AEDA). The extracts (1 µL) of the four samples (LHSMP, HHSMP, LHCHOC, HHCHOC) were injected in splitless mode into the injection port of an Agilent HP5890 gas chromatograph fitted with an ODO II odor port (SGE) and a polar ZB-wax column (Phenomenex, UK) (30 m × 0.25 mm i.d. × 0.25 µm). The carrier gas was helium at 2 ml/min with a 50:50 split between the odorport and the FID. After injection, the GC oven was held at 40 °C for 5 min, ramped at 5 °C/min to 250 °C and then held for 15 min. The effluent from the column was split 1:1, v/v, to an FID and a humidified sniffing port. Three experienced assessors evaluated each sample in duplicate, describing odors in their own words and recording the description alongside the retention time. Assessors were also asked to score the overall intensity of each odor using a 1-10 scale (where 1 = barely perceptible and 10 = overpoweringly strong). The modified frequency (%MF) was calculated according to Dravnieks.[16] All odors reported were detected by at least two assessors.

The flavor dilution (FD) factors of the odorants in the four samples were determined by AEDA. Extracts were diluted stepwise with diethyl ether (1: 2, v/v), and aliquots of the dilutions (1 µL) were evaluated by one assessor. A homologous series of *n*-alkanes C_5–C_{30} was analyzed under the same conditions to obtain linear retention index (LRI) values. Volatiles were identified by comparing the LRI value and odor description to those of an authentic standard, analyzed by GC-O under the same experimental conditions. In addition, the extract was sniffed on a DB5 column under similar conditions and the LRIs compared to those of authentic standards.

Gas Chromatography-Mass Spectrometry (GC-MS). SAFE extracts (1 μL) were analyzed in splitless mode on a DB-Wax column (Agilent) (30 m × 0.25 mm i.d. × 0.25 μm film thickness) using an Agilent 6890/5975 GC–MS system. The carrier gas was helium with a flow rate of 1 ml/min. The GC oven was held at 40 °C for 5 min, ramped at 5 °C/min to 250 °C and held for 15 min.

Mass spectra were recorded in electron impact mode at an ionization voltage of 70 eV and source temperature of 230 °C. A scan range of *m/z* 29-400 with a scan time of 0.69 s was employed and the data were controlled and stored by the ChemStation system. A homologous series of *n*-alkanes (C_5–C_{30}) was analyzed under the same experimental conditions to obtain LRI values. Volatiles were identified by comparing the mass spectrum and LRI value with those of authentic samples run under the same conditions. Each sample was analyzed in triplicate. Approximate relative concentrations were calculated by comparison of the peak areas against those of the internal standard, using a response factor of 1 for each compound.

Statistical analysis. SENPAQ version 3.2 (Qi Statistics, Reading, UK) was used to carry out two-way ANOVA on sensory profiling data where main effects were tested against the sample by assessor interaction. Multiple pairwise comparisons were done using the Fisher's least significant difference (LSD) test with the significance level set at $p<0.05$. The binomial test for probability was used to analyze the discrimination test data (Diff test version 2.1, StatBasics, Birmingham, UK). XLSTAT was used to carry our ANOVA on the GC-MS data.

RESULTS

Sensory Analysis. Two discrimination tests were carried out with an untrained panel of 50 assessors, to establish whether a difference was perceived between the two types of white chocolate. Using a triangle test, a significant difference between the samples was established where 26 out of 50 assessors correctly identified the different sample ($p = 0.005$). The 2-AFC test showed that HHCHOC was perceived to have "more caramel flavor" than LHCHOC, with 42 out of 50 assessors selecting the sample prepared with HHSMP ($p<0.0001$).

Having used discrimination testing to establish a significant difference between the model white chocolates produced using low and high heat SMP, sensory profiling was carried out with a trained panel to identify the specific attributes responsible for this difference.

Of 34 attributes describing the samples, five were found to be significantly different between LHCHOC and HHCHOC (Figure 2, see Table S2 for all attributes). Yellow color ($p<0.001$), overall flavor intensity ($p<0.01$), fudge flavor ($p<0.05$) and condensed-milk flavor ($p<0.05$) were rated significantly higher in HHCHOC, whereas hardness of bite was significantly higher ($p<0.05$) in LHCHOC. The yellow color of the HHCHOC reflected the fact that the HHSMP was also slightly yellow compared to the LHSMP, consistent with a greater thermal process and indicative of Maillard browning. The flavor attributes that were scored higher in HHCHOC were both heated notes, fudge and condensed-milk, which are generally associated with the Maillard reaction. Both the color change and the differences in flavor attributes are consistent with the fact that the SMP used to prepare the HHCHOC had received more thermal processing than that used for the LHCHOC.

Volatile compounds: GC-Olfactometry. Having established a sensory difference between LHCHOC and HHCHOC, the volatile profiles of the SMP and model white chocolate samples were analyzed and compared to determine the key compounds responsible for this difference. GC-Olfactometry (GC-O) analysis of the four extracts yielded 42 odor-active regions which were described by at least two out of three assessors (Table 2). Of these 42 odor-active regions, 34 were attributed to the corresponding odorant by running authentic reference compounds under the same analytical conditions, and matching both the LRI and odor description to those obtained during GC-O analysis. Short chain fatty acids were the major compounds identified in all samples, with butanoic acid showing the highest modified frequency (MF) overall. Other compounds with a high MF (≥ 40%) were furaneol (burnt sugar, candy floss), maltol (burnt sugar, sweet), 2-acetyl-1-pyrroline (popcorn, toasted), dimethyl trisulfide (pickled onions, cabbage), (*Z*)-4-heptenal (lamb fat, potato), 1-octen-3-one (mushroom, earthy), (*E,E*)-2,4-nonadienal (fried, hazelnut) and (*E,E*)-2,4-decadienal (nutty, fried). These compounds have all been previously identified in both SMP[17] and milk chocolate[1,2] by GC-O.

Short chain fatty acids have previously been identified as the most abundant volatile components in SMP.[14] This is consistent with our GC-O findings, as short chain fatty acids were detected in all four samples. Butanoic acid in particular was the only compound that was detected by all the assessors in all the extracts and the MF was > 80% for all samples. In milk, free fatty acids can be released through the hydrolysis of fat by lipases,[11,14] but high temperature will also enhance the hydrolysis of free fatty acids from the glycerol backbone.[18] Short-chain free fatty acids contribute cheesy, sweaty notes to the flavor profile, which can lead to rancid off-notes at high concentrations. However, the chocolate samples in this study did not receive high scores for cheesy odor or flavor attributes during sensory profiling (Table S2) and, although HHCHOC was scored higher than LHCHOC, the difference was not significant. This is consistent with the work on boiled milk reported by Pistokoulou et al.[13] who found several acids to have relatively high FD factors by GC-O, but they were present in the milk at concentrations below the reported odor threshold.

Products of lipid oxidation and degradation, such as aldehydes and ketones, were described as having green, mushroom, waxy, fatty and fried aromas. These compounds are often present at concentrations below the detection limit of the mass spectrometer, but can nevertheless be detected by assessors during GC-O because of their very low odor thresholds (e.g. the odor threshold of 1-octen-3-one in oil is 0.0001 mg/kg[19]). Of these compounds, 1-octen-3-one, (*Z*)-4-heptenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal had the highest MF. Identified previously as a primary odorant in milk products,[20] (*E,E*)-2,4-decadienal (nutty, fried) has also been shown to be an important odorant in milk chocolate.[2] Vazquez-Landaverde et al.[21] demonstrated a large increase in the total concentration of both aldehydes and ketones after UHT treatment of milk. Our results support these findings: the general trend within this group was for an increase in the high heat products. However, for some compounds, these differences decreased after processing into model white chocolate.

Sulfur-containing compounds, such as methional and dimethyl trisulfide, also have low odor thresholds. They were identified in all samples and had a higher MF in HHSMP and HHCHOC, compared to LHSMP and LHCHOC respectively. Al-Attabi et al. identified sulfur compounds as significant contributors to the cooked flavor of UHT milk.[22] During thermal processing of milk, the Strecker degradation of methionine forms methional,[23] which explains the higher scores for this compound in HHSMP. With further heating, methional is degraded to dimethyl disulfide[24] (via methanethiol), which is further converted to dimethyl trisulfide. During sensory profiling, the HHCHOC was scored significantly more highly than LHCHOC for “condensed-milk” flavor, and it is likely that methional and dimethyl trisulfide were contributors to this cooked flavor. Koyabashi et al.[12] reported that 2-methyl-2-furyl methyl disulfide and bis(2-methyl-3-furyl) disulfide contributed to brothy notes in HHSMP. The former was detected by GC-O in all four extracts with MF<30%, but this is one of few compounds where the MF was greater in the LHSMP compared to the HHSMP. Although present in the white chocolate extracts, no brothy notes were identified in the chocolate by the sensory panel and, in this case, these compounds are unlikely to be contributing to the difference in flavor of the two chocolates.

Maillard reaction products contributing cooked and caramel notes are the most likely cause of the flavor differences between LHCHOC and HHCHOC. Maltol, furaneol and 2-acetyl-1-pyrroline all had MF>40% and were detected in all four samples. Maltol and furaneol received higher MF scores in HHSMP compared to LHSMP and the same trend was observed in the corresponding chocolates. They both impart a sweet, caramel odor and this is consistent with the sensory results which showed a significant increase in fudge flavor and caramel flavor in the sensory profiling and discrimination tests respectively.

2-Acetyl-1-pyrroline (popcorn, toasted) is a potent aroma compound, which can be formed by the Maillard reaction of proline,[25] and has been identified extensively in basmati rice[26] as well as in UHT milk[27] and SMP.[11] There was a small difference in MF scores for 2-acetyl-1-pyrroline between heat treatments for SMP.

Other thermally-derived compounds, such as 2,3-butanedione (butter, creamy) and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) (curry, maple, burnt rubber), were also detected but showed much lower MF. In a study by Vasquez-Landaverde et al.,[21] 2,3-butanedione was one of the ketones that increased significantly between raw and UHT milk. In this study, it was difficult to draw conclusions about the levels of 2,3-butanedione as it is a highly volatile (boiling point 88 °C) and low molecular weight (86 g/mol) compound that is easily lost during concentration.

Volatile compounds: Aroma extract dilution analysis. AEDA is another technique which can be used to compare the relative intensity of aroma compounds within and between extracts. A single assessor was used for AEDA to compare the low and high heat samples

(Table 3) and confirm differences between products which had already been identified by three assessors using the GC-O technique discussed above. Although Ferreira et al.[28] have recommended the use of a larger pool of assessors and fewer dilutions (1:10) for AEDA, it was more practical to use small dilutions and a single assessor.

In general, the most persistent odor compounds in the milk powder extracts (FD 81) were those which also had a high MF. They included three fatty acids, acetic acid, maltol and furaneol as well as two unidentified compounds - one with a minty aroma (LRI 1704) and the other with a milky nutty aroma (LRI 1639). The lipid degradation products and the sulfur compounds tended to be less persistent by 1 or 2 FD factors. However those that persisted the longest in the chocolate extracts (FD 27), in addition to the acids, were the lipid degradation products ((*Z*)-4-heptenal and 1-octen-3-one), pyrazines and furaneol as well as one tentatively identified compound which eluted at the correct LRI (1509) for 2-(1-methylpropyl)-3-methoxypyrazine and imparted the green, potato and green pepper aroma typical of this compound. This may have been introduced into the system from the cocoa butter.

It is the difference between HH and LH which is important when accounting for the flavor differences between LHCHOC and HHCHOC. In the milk powder extracts, there were six compounds which were detected in the HHSMP but not in the LHSMP. Furthermore, there were 13 compounds that showed a difference in FD factor of at least 2 (representing at least a 1 in 9 dilution), nine of which were higher in HHSMP, confirming differences in MF discussed above.

A similar trend was found in the chocolate extracts, with nine compounds showing a difference in FD factor of 2 or more, all of which were higher in HHCHOC compared to LHCHOC. The difference between the furaneol FD factors for LHCHOC and HCHOC was 3 (1 in 27 dilution), consistent with the differences found in the GC-O and the increase in caramel and fudge notes detected in the HHCHOC by the sensory panels. Maltol showed a difference of 2 FD factors and was overall less persistent than furaneol. Trimethylpyrazine and 2,3-diethyl-5-methylpyrazine also had FD factors of 27 in the HHCHOC and persisted for two more FD factors compared to LHCHOC. Interestingly, these pyrazines had relatively low MF scores in the GC-O study, whereas 2-acetyl-1-pyrroline had MF>40% in the chocolate extracts, but was barely detected by AEDA. These could be due to assessor differences or could be indicative of the differences between the two GC-O techniques. Otherwise the results are fairly consistent between the two techniques. It is interesting that the unidentified aroma with a nutty, cooked milk, toasted and biscuit character which was prominent in the SMP, was barely detected in the chocolate and therefore unlikely to contribute to the flavor change.

Lipid degradation products are significant contributors to off-flavor in milk powder.[29] FD factors for these compounds were generally low in the chocolate extracts, except for (*E,E*)-2,4-decadienal, (*Z*)-4-heptenal and 1-octen-3-one (FD 27), which also had high MF scores during GC-O analysis. 1-Octen-3-one (earthy, mushroom) was identified in previous studies as one of the most significant off-flavors in skim milk powder,[17] formed as a result of light-induced oxidation, often during long-term storage of milk powder.[30] However the sensory profiling of the chocolate showed relatively low mean scores for cardboard odor (<9), which is a common descriptor for the oxidized off-flavor in milk caused by these compounds.[17]

Volatile compounds: GC-MS. Gas chromatography-mass spectrometry (GC-MS) was used to aid identification of compounds present in the samples and Table 4 lists the compounds identified. Fewer compounds were identified by GC-MS, compared to the GC-O. This demonstrates that many of the odor-active compounds were present at levels above the GC-odor detection threshold but below the detection limit of the instrument. Conversely, it was possible to identify some compounds that were not detected by GC-O analysis, were unlikely to be odor-active but provide additional evidence of, for example, greater Maillard activity in the more thermally processed samples.

Maillard-derived compounds were found in both low and high heat samples, but were shown to be consistently higher in the high heat samples, for both SMP and chocolate. Sugar degradation products, such as 2-furfural, 2-furanmethanol and 2,3-dihydro-3,4-dihydroxy-6-methyl-4*H*-pyran-4-one were all significantly higher in the HHSMP compared to the LHSMP, and although not all of these were detected in the chocolate, the same trend was observed for those that were. 5-(Hydroxymethyl)furfural (HMF) is often used as a marker of thermal processing in milk,[31] however there was not a significant difference in the amount of HMF between the two SMPs and therefore it cannot be considered to be a good marker of heat treatment in this case. This supports previous work by Berg and van Boekel,[32] which demonstrated that HMF is not formed in significant concentrations in milk (<400 µmol/L) after 10 min heating at 150 °C or 20 min at 140 °C.

2-Furfural can be formed via the formation of Amadori compounds, from the reaction of lactose and lysine, or as a result of the isomerization of lactose to lactulose.[33] Similarly, 2-furanmethanol is likely to be formed from the thermal breakdown of lactose. Although described as having a sweet, nutty odor, the odor detection thresholds of 2-furfural and 2-furanmethanol in water are 2000 and 3000 µg/kg respectively.[34] As a result, the concentrations were likely to be too low to contribute to the aroma profile of these samples, but the increase in the high heat samples is further evidence of enhanced Maillard activity. These compounds have not been identified before as odour-active in milk chocolate.[1,2]

DISCUSSION

The directional discrimination test with naïve panellists showed that the chocolate prepared from the HHSMP had more caramel/fudge character ($p<0.0001$), and sensory profiling with an expert panel confirmed the increase in the intensity of both the fudge flavor ($p<0.05$) and the condensed milk flavor ($p<0.05$). GC-MS and GC-Olfactometry were carried out in order to understand what was driving these differences in perception. The aroma of the white chocolate undoubtedly results from the combination of many of the compounds identified. However, those most likely to compounds to contribute to the change in aroma when HHSMP was used are likely to be those that were detected consistently by GC-O, had relatively high %MF scores (Table 2) and high FD factors (Table 3). More importantly, they are those where there was a significant difference observed between the HHCHOC and the LHCHOC, either in %MF, FD or both. Finally, the compounds responsible are likely to have aroma characteristics similar to those described by the panellists. On these grounds, the acids, which were amongst the highest scoring compounds, were ruled out as they tended not to increase substantially in the HH products, the cheesy notes were not detected by the panel and previous work has shown that despite the high FD values, they are usually present at concentrations below their odour threshold[13]. The high scoring lipid-derived compounds were discounted on the grounds that the aroma characters were uncharacteristic of the perceived sensory difference. The sulfur compounds (methional and dimethyl trisulfide) scored very highly and, although their aroma is also uncharacteristic of those used by the panellists, they have been shown to contribute to the cooked notes in UHT milk,[22] and could be contributing to the condensed-milk flavor which was significantly higher in HHCHOC. The group of Maillard-derived compounds are those which are likely to be contributing to the increase in fudge and caramel aroma. Maltol, furaneol, 2-acetyl-1-pyrroline all had high %MF and high FD factors particularly in the HH products. Maltol and furaneol impart sweet and burnt sugar notes which both persisted for two or more FD factors in HHSMP or HHCHOC, compared to

LHSMP and LHCHOC respectively. They are likely to contribute to the perceived increase in fudge and caramel notes as well as providing some sweet character to the condensed milk notes. 2-Acetyl-1-pyrroline imparts a more roasted popcorn note which might contribute to the toasted character in the fudge notes. Trimethylpyrazine and 2,3-diethyl-5-methylpyrazine did not have high %MF scores, but had high FD factors which were higher in the HH products. It is a combination of these Maillard-derived compounds which is likely to be driving the difference between the HHCHOC and the LHCHOC. This is entirely consistent with the fact that the difference between them is a 5 min heat treatment of the milk at 125 ºC prior to spray-drying, conditions which will promote the Maillard reaction in the HH products. The sensory results demonstrate that this difference carries through to the white chocolate where significant differences in flavor were perceived.

Furaneol has a low odor detection threshold of 10 μg/kg,[35] but was not detected by GC-MS in the chocolate extracts. On the other hand, the odor detection threshold of maltol is much higher and reported values vary from 9000 μg/kg[36] to 35000 μg/kg.[37] From addition of a known amount of maltol-(*methyl*-d_3) to the DEE extracts prior to SAFE extraction, the concentration of maltol in the model white chocolate prepared from low and high heat SMP was found to be 122 and 315 μg/kg respectively. These concentrations are well below the reported thresholds, but the reported threshold values were determined in water whereas chocolate has a continuous fat-phase and a very low water content. The threshold and flavor release of maltol from the chocolate matrix will be very different to that of water, as maltol is relatively hydrophilic (Log P = 0.07±0.282 calculated from Advanced Chemistry Development (ACD/Labs) Software V11.02). Without more appropriate threshold data, the relative contribution of maltol and furaneol to the caramel note cannot be determined.

Maltol is formed from the Maillard reaction of lactose[38, 39] and it has been suggested that it can be formed during the conching of chocolate. Counet et al.[3] found much higher concentrations of maltol in conched dark chocolate (4.2 and 28.4 mg/kg) and demonstrated a six fold increase during conching. However, typical conching temperatures for dark chocolate are higher than that used for the white chocolate in this study (70 - 80 °C compared to 50 °C) as there is less need to avoid browning in milk chocolate and dark chocolate. Liu et al[2] found similar a concentration in dark chocolate (1.9 mg/kg) but less in milk chocolate (715 μg/kg), more in line with the quantities found in white chocolate. Previous work in our laboratory[40] showed no significant difference in maltol concentration between the model white chocolate analyzed before and after conching. This confirmed that these key Maillard-derived compounds were formed during the production of the milk powder, and not during chocolate processing.

Overall, results from this study demonstrate that the SMP manufacturing process can influence the flavor profile of model white chocolate. Many thermally-derived compounds were present at significantly higher concentrations in HHSMP, and were shown to be formed during the heating step traditionally carried out before the concentrated milk is spray-dried. This flavor difference carries over into the white chocolate which was prepared from the corresponding SMPs. The most significant flavor differences between white chocolate produced from LHSMP or HHSMP are likely to be attributed to the Maillard-derived compounds (maltol, furaneol, 2-acetyl-1-pyrroline, trimethylpyrazine and 2,3-diethyl-5-methylpyrazine) and sulfur compounds (methional and dimethyl trisulfide). This understanding of flavor generation in SMP is important for confectionery manufacturers to maintain, or manipulate, the flavor of their products.

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ASSOCIATED CONTENT

Sensory reference materials are listed in Table S1, and Table S2 shows mean panel scores (n = 9) for all sensory attributes of two types of white chocolate produced using skim milk powders of different heat treatments. This material is available free of charge via the Internet at http://pubs.acs.org.

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FIGURE CAPTIONS

Figure 1 Schematic diagram of the manufacture of the slim milk powders

Figure 2 Sensory attributes showing a significant difference between two white chocolates prepared using skim milk powders produced with different heat treatments – high heat (HHCHOC) and low heat (LHCHOC). Intensity is the mean score of two replicate assessments for each assessor (18 replicates in total). * = Probability, obtained from ANOVA, that there is a difference between means; ns = no significant difference between means ($p>0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level. Error bars extend +/- one half of the least significant difference (LSD)

Table 1 Composition of liquid milk measured during skim milk powder production

composition (%)	raw whole milk	raw skim milk	pasteurized milk	concentrated milk
fat	4.46	0.07	0.08	0.24
protein	3.26	3.15	3.1	9.6
lactose	4.62	4.41	4.36	13.5
total solids	12.3	7.5	7.41	23.2

Table 2 Odor-active volatiles in high heat skim milk powder (HHSMP), low heat skim milk powder (LHSMP), high heat model white chocolate (HHCHOC) and low heat model white chocolate (LHCHOC)

Linear Retention Index[a]				odor description	identification	freq.[b]	modified frequency [MF(%)][c]			
Wax expt	Wax au	DB5 expt	DB5 au				LH SMP	HH SMP	LH CHOC	HH CHOC
short chain fatty acids										
1445	1435	nd	577	vinegar, acidic	acetic acid	13	32	32	29	23
1562	1568	nd	757	sweat, cheesy	2-methylpropanoic acid	4	11	17	nd	nd
1608	1603	nd	775	cheese, acid	butanoic acid	24	91	91	81	82
1661	1645	857/836	845/839	sharp, tangy, acidic, cheese	2/3-methylbutanoic acid	22	74	72	63	71
1733	1712	nd	897	sweaty, cheese, acidic	pentanoic acid	18	58	60	22	45
1833	1821	nd	984	sweaty, cheesy, tangy	hexanoic acid	19	78	84	49	44
lipid-derived aldehydes and ketones										
1054	1063	808	802	green, grass	hexanal	18	30	42	39	47
1164	1171	nd	903	fruity, berries	heptanal	8	20	22	13	22
1229	1225	909	904	lamb fat	(*Z*)-4-heptenal	20	42	51	42	53
1272	1283	988	978	mushroom, earthy	1-octen-3-one	23	55	62	55	57
1434	1408	1075	1063	fatty, waxy	(*E*)-2-octenal	5	17	21	nd	nd
1488	1478	1203	1209	sheets, waxy	decanal	12	20	45	26	27
1517	1512	1159	1168	fatty, waxy	(*E*)-2-nonenal	14	37	44	28	39
1569	1567	1111	1117	violet, floral	(*E,E*)-2,4-octadienal	8	nd	20	24	28
1683	1680	1233	1228	fried, hazelnut	(*E,E*)-2,4-nonadienal	16	45	53	42	43
1738	1728	1379	1368	coriander	(*E*)-2-undecenal	9	14	25	26	26
1794	1788	1325	1327	nutty, fried	(*E,E*)-2,4-decadienal	15	41	51	47	47
sulfur compounds										
1361	1354	975	984	pickled onions, drains	dimethyl trisulfide	22	51	70	67	71
1438	1432	919	912	cooked, savory, chips	methional	12	30	35	27	34
1655	1653	1181	1184	savory, beefy	2-methyl-3-(methyldithio)furan	9	29	22	24	26
Maillard reaction products										

962	956	<600	600	butter, creamy	2,3-butanedione	5	16	16	nd	8
1320	1322	939	929	basmati, toasted	2-acetyl-1-pyrroline	22	65	69	45	54
1945	1932	1128	1126	burnt sugar, caramel, sweet	maltol	20	58	74	41	44
2009	1998	1136	1066	sweet, strawberry, caramel	furaneol	22	59	70	45	51
2166	2222	1164	1068	maple, curry	sotolon	5	13	9	nd	nd
1398	1386	1007	1008	biscuit, peanuts	2,3,5-trimethylpyrazine	12	34	30	26	24
1474	1469	1157	1157	fried, hot oil, potato	2,3-diethyl-5-methylpyrazine	9	11	nd	27	30
lactones										
1932	1925	nd	1266	coconut, milky	γ-octalactone	5	nd	17	8	13
2131	2134	nd	1478	cooked milk, sweet	γ-decalactone	6	16	9	16	13
2416	2413	nd	1507	condensed milk, creamy	δ-dodecalactone	5	12	25	nd	16
unidentified and tentatively identified aromas										
980	-	nd	-	sulfurous, rotting	unknown	6	16	16	13	11
1372	-	995	983	mushroom	1-octen-3-ol	11	12	29	23	29
1404	-	1289	-	liquorice, creamy	unknown	10	nd	nd	25	33
1417	-	nd	-	green, earthy	unknown	6	nd	nd	17	27
1421	-	nd	-	cooked, burnt toast, cardboard	unknown	11	32	35	25	17
1509	1510	nd	1181	green, potato, green pepper	2-isobutyl-3-methoxypyrazine	13	25	nd	44	52
1607	1584	1319	1305	hot, dry	undecanal	7	14	17	18	20
1639	-	nd	-	nutty, cooked milk, biscuit,	unknown	15	34	35	37	40
1704	-	nd	-	minty	unknown	17	33	39	45	45
1842	-	nd	-	medicinal	unknown	10	nd	28	26	28
1986	-	nd	-	hot, dry, waxy	unknown	8	11	13	26	18
2070	2032	nd	1171	acidic, sweat, cheese	octanoic acid	14	43	52	14	22

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[a] Linear retention index of aroma by GC-O (expt) or of authentic aroma compounds by GC-O (au) determined on either a ZB-Wax or DB5 column, calculated from a linear equation between each pair of straight chain alkanes C_5–C_{30}

[b] Detection Frequency (freq): total number of times odorant was detected (maximum = 24)

[c] Modified frequency (%MF) was calculated with the formula proposed by Dravnieks[16]: $MF(\%) = \sqrt{F(\%) \times I(\%)}$, where $F(\%)$ is the detection frequency expressed as a percentage and $I(\%)$ is the average intensity expressed as a percentage of the maximum intensity. nd = not detected

Table 3 Aroma extract dilution analysis (AEDA) of extracts of high heat skim milk powder (HHSMP), low heat skim milk powder (LHSMP), high heat white chocolate (HHCHOC) and low heat white chocolate (LHCHOC)

odorant	LRI[a]	FD factor[b]			
		LH SMP	HH SMP	LH CHOC	HH CHOC
short chain fatty acids					
acetic acid	1445	9	81	9	9
2-methylpropanoic acid	1562	1	9	-	-
butanoic acid	1608	27	81	9	27
3- and 2-methylbutanoic acid	1661	27	81	9	27
pentanoic acid	1733	9	1	3	9
hexanoic acid	1833	9	9	9	27
lipid-derived aldehydes and ketones					
hexanal	1054	-	3	1	3
(*Z*)-4-heptenal	1229	1	9	3	27
1-octen-3-one	1272	1	9	3	27
decanal	1488	3	3	3	9
(*E*)-2-nonenal	1517	1	3	3	3
(*E,E*)-2,4-octadienal	1569	-	1	1	3
(*E,E*)-2,4-nonadienal	1683	9	1	-	-
(*E*)-2-undecenal	1738	1	9	-	3
(*E,E*)-2,4-decadienal	1794	27	27	1	9
sulfur compounds					
dimethyl trisulfide	1361	1	9	9	27
methional	1438	1	27	1	1
Maillard reaction products					
2-acetyl-1-pyrroline	1320	3	9	1	1
maltol	1945	9	81	1	9
furaneol	2009	9	81	1	27
sotolon	2166	9	3	1	1
trimethylpyrazine	1407	-	1	3	27
2,3-diethyl-5-methylpyrazine	1474	9	27	3	27
lactones					
γ–decalactone	2131	1	3	-	3
δ–dodecalactone	2416	-	1	9	9
unidentified and tentatively identified aromas					
1-octen-3-ol	1372	3	1	-	3
2-isobutyl-3-methoxypyrazine	1509	-	-	1	27
unknown (nutty, cooked, milky)	1639	-	81	-	1
unknown (minty)	1704	9	81	1	9
octanoic acid	2070	9	81	3	9

[a] Linear retention index on ZB-Wax column, calculated from a linear equation between each pair of straight chain alkanes C_5–C_{30}

[b] Flavor dilution (FD) factor: the dilution at which the odorant was no longer detected by GC-O. Serial dilutions were prepared from the initial extract at a ratio of 1:3 in ether, results from one assessor

Table 4 GC-MS analysis (data expressed in ug/kg relative to the internal standard) carried out on extracts of high heat skim milk powder (HHSMP), low heat skim milk powder (LHSMP), high heat white chocolate (HHCHOC) and low heat white chocolate (LHCHOC)

LRI [a]	ID [b]	compound	Relative concentration (µg/kg) [c]					
			in skim milk powders			in model white chocolate		
			LHSMP	HHSMP	S [d]	LHCHOC	HHCHOC	S [d]
fatty acids								
1466	A	acetic acid	939 (110)	1380 (24)	**	3480 (1410)	16200 (9640)	ns
1550	A	propanoic acid	262 (62)	425 (60)	***	413 (42)	917 (133)	**
1566	A	2-methylpropanoic acid	208 (125)	379 (282)	ns	nd	nd	
1635	A	butanoic acid	12300 (4680)	16900 (4590)	ns	1940 (666)	3340 (1010)	ns
1740	A	pentanoic acid	390 (143)	460 (182)	ns	471 (132)	1010 (524)	ns
1845	A	hexanoic acid	17800 (13900)	22900 (7440)	ns	1030 (598)	1370 (78)	ns
2056	A	octanoic acid	13800 (11300)	17900 (7190)	ns	873 (564)	726 (469)	ns
2162	A	nonanoic acid	396 (135)	994 (1040)	ns	594 (201)	642 (389)	ns
2268	A	decanoic acid	4050) (178	5350 (553)	ns	461 (14)	1020 (395)	ns
Maillard reaction products								
1449	A	2-furfural	872 (324)	1560 (477)	*	nd	nd	
1521	A	benzaldehyde	548 (264)	820 (190)	*	144 (47)	867 (147)	**
1661	A	2-furanmethanol	5850 (340)	9140 (2050)	**	66 (22)	393 (64)	**
1963	A	maltol	12000 (1300)	20200 (5150)	**	201 (29)	1540 (273)	**
2014	A	furaneol	717 (141)	1060 (255)	**	nd	nd	
2099	A	norfuraneol	905 (176)	1500 (483)	*	nd	nd	
2316	B	2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	598 (5410)	1220 (2930)	*	9 (6)	42 (12)	*
2500	A	5-(hydroxymethyl)furfural	833 (335)	1260 (429	ns	18 (3)	45 (6)	**
1479	A	tetramethylpyrazine	nd	nd		58 (29)	125 (63)	ns
lactones								
1966	A	δ-octalactone	nd	nd		257 (19)	624 (137)	*
2191	A	δ-decalactone	nd	nd		1240 (699)	2360 (947)	ns

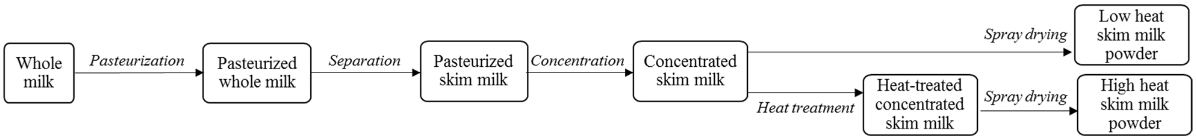
2377	A	γ-dodecalactone	nd	nd		30 (3)	64 (8)	**
2429	A	δ-dodecalactone	nd	nd		370 (46)	583 (122)	*
oxidation products								
1372	A	2-nonanone	nd	nd		212 (37)	581 (102)	**
1376	A	nonanal	159 (76)	278 (159)	ns	1020 (172)	1750 (765)	ns
1901	B	dimethyl sulfone	696 (343)	626 (112)	ns	199 (75)	709 (30)	***

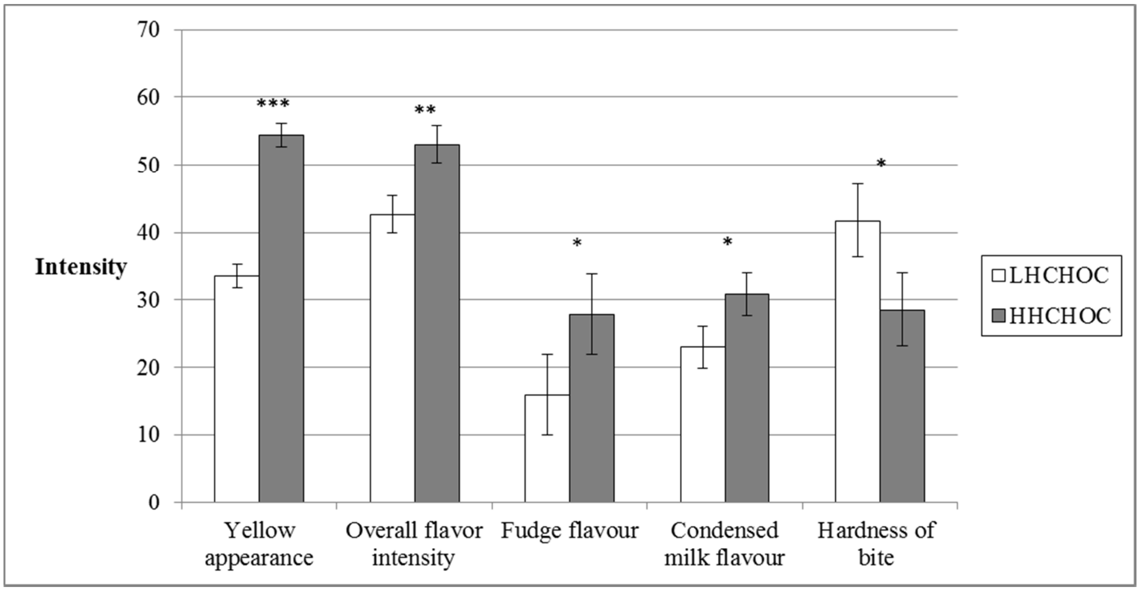
[a] Linear retention index on ZB-Wax column (30m), calculated from a linear equation between each pair of straight chain alkanes C_5–C_{30}.

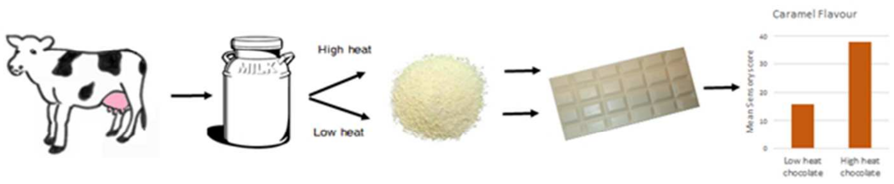
[b] Identity of compounds: A = confirmed by comparison of mass spectrum and LRI with those of authentic compounds, B = comparison of mass spectrum with NIST11 library

[c] Relative concentration = peak area of compound × concentration of internal standard (ISTD) / peak area of ISTD, nd = not detected. ISTD: 30 μL 2-methyl-3-heptanone (6.18 μg/25 mL) in methanol

[d] S: Significance of samples; Probability, obtained from ANOVA, that there is a difference between means; ns = no significant difference between means ($p>0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level.


Whole milk
Pasteurization
Pasteurized whole milk
Separation
Pasteurized skim milk
Concentration
Concentrated skim milk
Spray drying
Low heat skim milk powder
Heat treatment
Heat-treated concentrated skim milk
Spray drying
High heat skim milk powder


70
60
50
40
30
20
10
0
Intensity
***
**
*
*
*
Yellow appearance
Overall flavor intensity
Fudge flavour
Condensed milk flavour
Hardness of bite
□LHCHOC
■HHCHOC



121x29mm (150 x 150 DPI)