The Effect of Sampling Methods on Particle Size Distributions in Dry Whey Protein Concentrate (WPC)

Even Gausemel[1,2], Anne-Grethe Johansen[1,2], Elling-Olav Rukke[1], Camilla Elise Jørgensen[2], Kristian Hovde Liland[3] and Reidar Barfod Schüller[1]

[1]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1433 Ås, Norway
[2]TINE SA R&D, Bedriftsveien 7, 0950 Oslo, Norway
[3]Faculty of Science and Technology, Norwegian University of Life Sciences, P.O. Box 5003, N-1433 Ås, Norway

ABSTRACT

Whey Protein Concentrate 80 (WPC80) is a frequent ingredient in industrial food production. This ingredient is often sold in sacks of 20 kg or more. Samples are taken from WPC80 to evaluate quality as well as chemical, physical, and functional properties. When sampling for analysis of functionality it is important to choose a sampling method which yields representative samples. Bulk sampling methods for WPC80 sacks must address heterogeneity, e.g., different particle size distributions caused by segregation. WPC80 at the top of the sack may have different properties than at the bottom.

This introductory study on commercially available WPC80 aimed to determine if there were significant differences, in terms of Particle Size Distribution (PSD) responses, when comparing samples from two sampling methods. Further if there were significant differences between arbitrarily chosen sample groups from each sampling method. These quantitative research questions are relevant when choosing sampling methods, e.g., for quality evaluation operations at a production site for dairy ingredients or for scientific evaluation of powder properties.

Powder from one 20 kg sack of WPC80 was split into two 10 kg batches using a riffle splitter (RT 12.5. Retsch). Two sampling methods were compared: In one sampling method a 10 kg batch were split into suitable sample size using a single riffle splitter in multiple steps. The other 10 kg batch was manually mixed before grab sampling (a manual sampling operation). The particle size distributions were measured using a Malvern Mastersizer 3000 unit. Responses of interest were related to particle diameters calculated with Mie theory. Analysis of Variance, Tukey's pairwise comparisons and Student's t-test were used to evaluate the data.

Results showed significant differences between PSD responses, when comparing the two sampling methods. Use of a riffle splitter with multiple splitting steps yielded more representative samples, relative to the manual mixing and grab sampling method. Appropriate sampling method choices are relevant when a representative particle size distribution is required, e.g., in sensitive powder rheology measurements.

INTRODUCTION

Spray drying is a common production method to produce WPC80. In this process the liquid concentrate feed is turned into solid powder particles when exposed to a hot air environment[1]. During spray drying the liquid material is sprayed into droplets of different sizes, which are quickly dried[2], becoming primary particles. The primary particle size is influenced by several parameters. Spray drying conditions and the type of atomizing equipment are examples of groups of parameters[1]. WPC80 from spray drying are typically agglomerates of primary particles. Agglomerating the powder increases the particle size[1,3,4].

Food agglomerates are described as fragile and brittle[1,3]. Collisions between powder agglomerates, container walls and agitating instruments can cause shear, compression and/or mechanical impact. This can lead to agglomerates breaking, and dairy powder particles typically break into fine particles[1,3,5]. Shear motion and vibration in the powder may also cause segregation[6,7]. Powder breaking and segregation activity occur during the production and transport of dairy powders[1,5,3,4,6]. In top-to-bottom segregation, particles of different size can move to the bottom or to the top. One example mechanism is sieving segregation. Sieving segregation can occur when granular materials are exposed to vibration or shear motion. A common viewpoint is that a wide particle size distribution increases the likelihood for segregation[6].

Agglomeration of dairy powders can improve both rehydration and flowability properties[4], assuming that the agglomerates are kept intact. Different particle size distributions in powder samples, influenced by powder breaking, can be expected to influence analysis of physical properties and functionalities, such as powder flowability in agglomerated powders[1,3,5,4].

Representative sampling is mass reduction where heterogeneity is counteracted[8]. In other words, sampling is the collection of a smaller mass from a larger one. Representative sampling operations ensures that the analysed smaller sample(s) represents the larger mass. Mixing the bulk before sampling is advised, but mixing has limits, especially where segregation[6] or particle breakage[1] is expected[8].

In this study two different WPC80 sampling methods were investigated. Powder from one 20 kg sack of WPC80 was split into two 10 kg batches using a riffle splitter (RT 12.5. Retsch). Two sampling methods were compared: In one sampling method a 10 kg batch were split into suitable sample size by using a single riffle splitter in multiple steps. The other 10 kg batch was manually mixed before grab sampling. Grab sampling is a type of discrete sampling operation where a sampling instrument is used. It is the direct and manual extraction of one or more small amounts, as a sample[8]. Sampling methods and groups of samples within the same sampling method were compared. The aim was finding differences in PSD responses and to identify which sampling method is most appropriate when preparing a set of samples and arbitrarily drawing samples, for analysis of particle size distributions.

MATERIALS AND METHODS

WPC80, sampling methods A and B

Commercially available WPC80 from TINE SA, in a 20 kg sack, was brought to a room with controlled air temperature and relative humidity. It was expected that 20 kg of powder were heterogenous in terms of PSD. A 12.5 mm slot, size static riffle splitter with 18 slots (Sample splitter RT 12.5, Retch GmbH, Germany, 2021.) was used to split 20 kg WPC80 into two batches of ~10 kg. Energy, fat, carbohydrate, and protein values for commercially available TINE SA WPC80 are described in **Table 1**.

TABLE 1: Nutritional values for commercially available WPC80 as declared by TINE SA

Nutritional value	/100g
Energy	**1650 kJ (390 kcal)**
Fat	6.5 g
Carbohydrate	8 g
Protein	77.4 g

The two sampling methods are illustrated in **Fig. 1.** In sampling method A, the size of the sample was reduced from ~10 kg to ~10 g, using a riffle splitter. This was done through a total of eleven splitting steps. After each splitting operation, half of the divided WPC80 amount was set aside. This powder was either meant for storage as excess powder, or for further splitting. Sixteen samples with a weight of ~10 grams were prepared, and all samples were tagged with sample order.

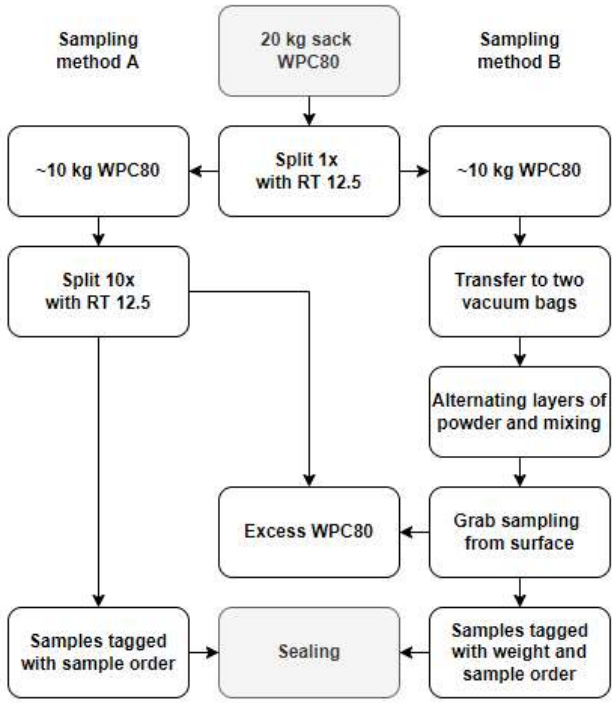

FIGURE 1: Method A left and method B right side

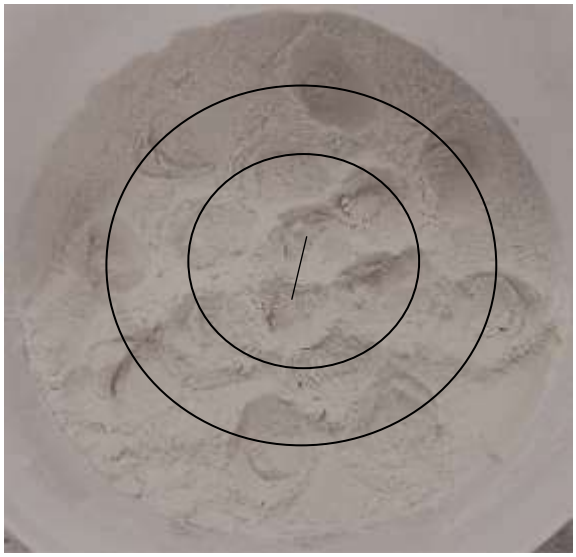

FIGURE 2: From sampling method B. Illustration of the pattern symmetry for grab sampling

Sampling method B was meant to represent manual mixing and sampling operations when multiple splitting steps with a riffle splitter is not feasible. In method B (**Fig. 1.**) the first and second parts of the 10 kg powder batch was temporarily stored in separate, sealed plastic vacuum bags, without use of vacuum (Henkelman vacuum systems 300 II, S.nr. 30809681, Henkelman bv., Netherlands, 1998). The powder from these bags were later alternatingly layered in a 30 L cylindrical plastic container. For every two layers the powder was thoroughly mixed by manual mixing, with a 1 L plastic container. The powder was manually mixed a final time when all powder was layered. This mixing was expected to expose the powder to shearing when the 1 L container was run through the powder. Shearing was expected to increase the breaking of agglomerated WPC80 particles. It was expected that WPC80 in sampling method B were still heterogenous in terms of PSD, after manual mixing.

At the time of grab sampling for method B, the top of the powder volume was made roughly flat. Sixteen samples were extracted from the surface with a metal spoon and weighed. Two samples were taken from the centre of the surface. Eight samples were taken closer to the edge and six samples from a middle concentric ring (**Fig. 2.**). The spoon was used to extract sample from a roughly round hollow and not only from the top layer.

All powder samples where temporarily stored in labelled, sealed vacuum bags in a climate-controlled chamber (HPP750 eco, Memmert GmbH, Germany, 2021) at 19°C. The vacuum bags were not exposed to vacuum. All samples were protected from light and moisture. The order of the samples was randomized in such a way that all samples were analysed in a random order.

Analysis and experimental set-up

This study was designed to avoid other differences between datasets than the factor of sampling method. A Malvern Mastersizer 3000 (S.nr. MAL1083189, Malvern, UK, 2013) equipped with an Aero S dry powder disperser was used to analyze particle size distributions in samples. Settings: Particle absorption index 0.005. Refractive index 1.461. 90% feed rate. 40 second measuring time per sample. Dispersion at 1.5 bar pressurized air.

The responses of interest were the diameter responses Dv 10, Dv 50 and Dv 90, describing three points in the PSD volume distribution. Dv 50 is the median of the volume distribution. In addition, volume mean diameter D[4,3] and Sauter mean diameter D[3,2] was evaluated. Sauter mean, a surface weighted mean diameter value, is more sensitive to the presence of fine particles in the particle size distribution[1,3].

Data analysis

Data from Malvern Mastersizer was evaluated with Malvern panalytical software, to ensure that the quality of the analyses was good. The final datasets were analysed in R 4.1.3 with Student's t-test or ANOVA with Tukey's pairwise comparisons.

The Tukey pairwise comparisons were applied to compare subgroups within sampling method A and B. The meaning of subgroup was that there were smaller groups of arbitrarily chosen samples, within the original set of sixteen samples. Each subgroup was made up of three samples with separate subgroups per sampling method. Each original set of sixteen samples was given a randomized order before it was broken down into subgroups. This was done to avoid any time dependent trends from the particle size distribution analysis.

To improve the chance of finding pairwise differences, the randomizing of the order before subgrouping and Tukey's pairwise comparisons were done three times per original set of sixteen samples. Each time one sample was excluded (never the same sample) to obtain five groups of three samples each. Then all subgroups were compared, per sampling method and for each of the five PSD responses.

RESULTS

Comparisons of the two sampling methods (**Fig 3.** and **Table 2.**) and comparisons of subgroups (n=3) both gave significant results.

Student's t-test null hypothesis was: True difference in mean values between method A and method B is equal to 0. All five PSD response mean values are significantly higher for sampling method A than for B (**Fig. 3.**).

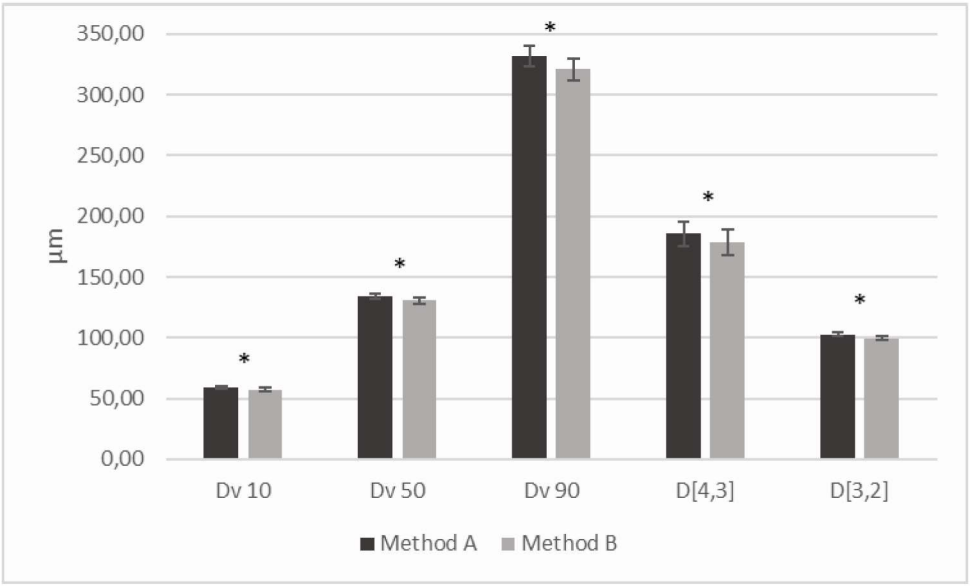

FIGURE 3: Mean Dv 10, Dv 50, D[4,3] and D[3,2] values from WPC80 samples. Each response mean value is connected to either sampling method A or B, as described in the figure caption. The method A samples were compared to method B samples, per PSD response. The values from different responses were not compared. The symbol * shown in the top of columns describes significant differences (P<0,05) between mean values (n=16). The error bars indicate standard deviations for each method-response combination

TABLE 2: P-values from Student's t-test comparisons of sampling methods A and B. Five responses

Response	P-value (n=16)
Dv 10	$7.39e^{-06}$
Dv 50	$1.07e^{-06}$
Dv 90	0.0005
D[4,3]	0.04
D[3,2]	$2.40e^{-06}$

In most cases the comparison of subgroups of three samples gave no significant differences. Comparison of subgroups from sampling method A did not give any significant differences. (Results not shown.) For sampling method B there were significant differences when analysing three of five responses. For all significant results, the subgroup with the highest mean value was different from either of the two subgroups with lowest mean values.

Significant differences between response values from, Tukey's pairwise comparisons, are shown in **Fig. 4.** This figure show results from comparisons of first randomized order subgroups, from sampling method B. Because the randomized order of samples was the same for all responses, all significant differences occurred between subgroups 2., 3. and 4. Subgroup 2. has a significantly higher value than subgroups 3. and 4. This is true for responses Dv 10, Dv 50 and D[3,2]. No significant differences were found between subgroups from the second or third randomizations of method B samples.

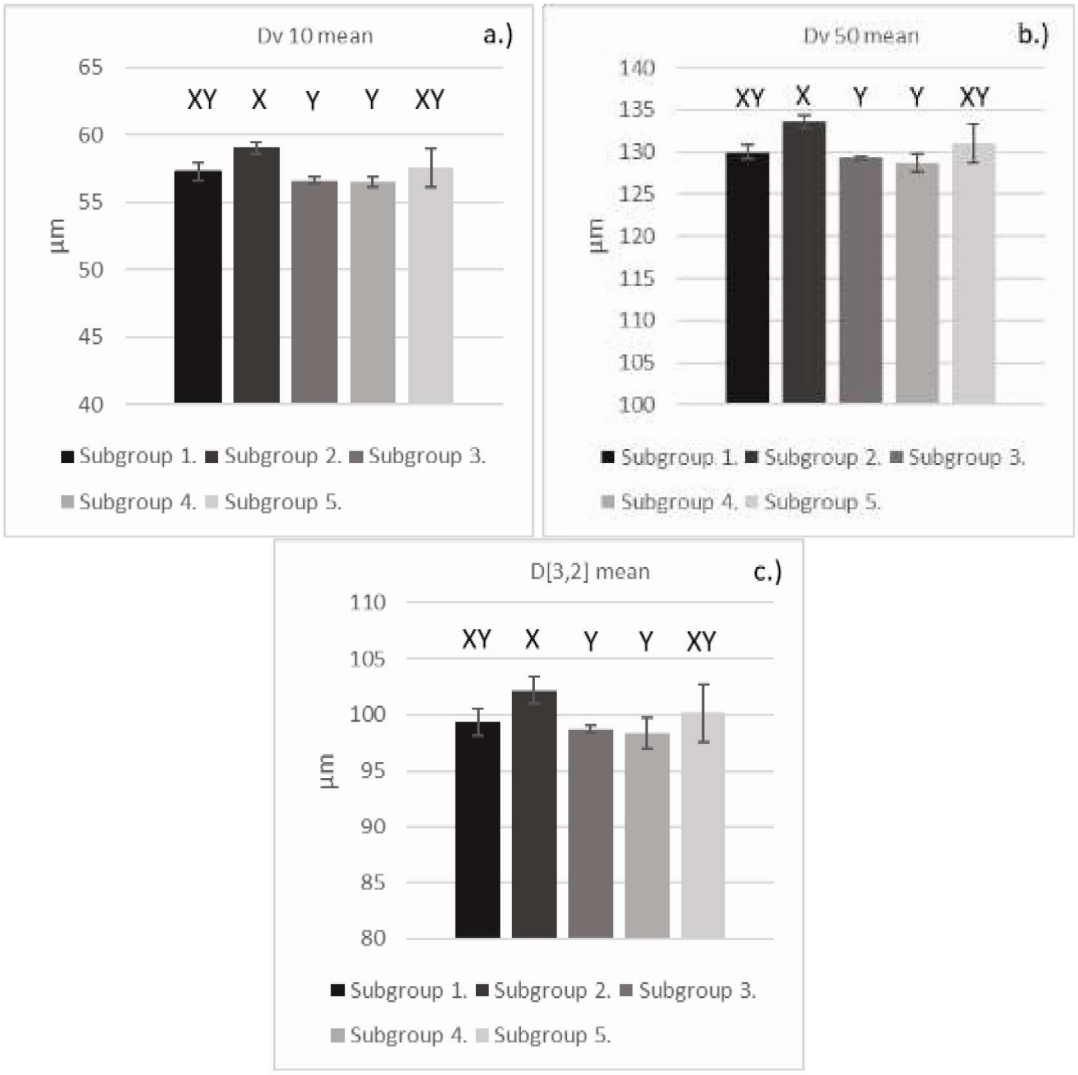

FIGURE 4: Each subgroup 1.-5. represents the same three arbitrarily chosen samples in each of the three graphs. The five subgroups (n=3) in each graph were compared. The letters X and Y, given at the top of the columns, indicate Tukey groups. Columns with no shared letters indicate significant differences between mean values. The responses are a.) Dv 10, b.) Dv 50 and c.) D[3,2]. The Y axis has been truncated to emphasise differences

DISCUSSION

The results indicate that the sampling methods yield significantly different results in terms of the chosen PSD responses. Furthermore, the results shows that sampling method B gives significantly different response values (Dv10, Dv50, D [3,2]) between subgroups. This indicates that the samples from this method are less representative, for these three responses, compared to method A. The results may be influenced by heterogeneity in the ~10 kg batch in method B, at the time of sampling. Heterogeneity in terms of PSD, from the 20 kg sack, may have been counteracted to a higher degree using the riffle splitter in method A.

Petersen, Dahl and Esbensen (2004) compared seventeen field and/or laboratory instruments for mass reduction in sampling. They claimed that riffle splitting is the most well-founded method for mass reduction[5,7]. This study on WPC80 sampling methods indicates that static riffle splitter is an appropriate sampling tool for this type of powder and sack size. Furthermore the riffle splitter gives more representative samples than the manual mixing and grab sampling method. This is in line with what is shown by Petersen, Dahl and Esbensen[7].

If the manually mixed 10 kg WPC80 batch (method B) was heterogenous this could impact the difference between mean values from sampling methods A and B. For example, if the surface of the 10 kg had relatively shorter mean diameter values because more fine particles were present. The heterogeneity in 10 kg could be the result of particle breakage, segregation, or insufficient counteracting of heterogeneity from the sack, with the manual mixing method. Use of the riffle splitter in method A, as an alternative to manual mixing may also have caused some breaking and/or segregation, but it is expected that the method counteracted the heterogeneity from sack.

The differences between subgroups were found for the lower diameter values Dv 10 and Dv 50, as well as Sauter mean D[3,2]. These responses could have been influenced by different extent of relatively large particles/agglomerates breaking into fine particles. A relatively larger amount of manual mixing could partially explain lower mean particle diameters in samples from method B, compared to samples from method A. If it is assumed that mean values from sampling method A have a PSD which is more representative for the PSD of the WPC80 sack, it is possible to infer more about sampling method B. Such an assumption is reasonable because no significant increase in particle sizes is expected during sample preparation in a controlled environment. The difference in responses indicates which group of powder samples are more different from the unopened WPC80 sack. WPC80 sampled with method B can be described as less representative of the 20 kg sack, following this assumption.

CONCLUSION

Particle size measurement results in this study show that there are significant differences between using multiple step riffle splitting (method A) and manual mixing followed by grab sampling (method B). This was shown for WPC80. Method B gave a significantly different result for all five responses. Significant differences between subgroups of samples using method B indicated that manual mixing and grab sampling yielded samples that were less representative than samples from method A.

If the aim of sample preparation is to yield similar and representative samples in terms of particle size distributions, the multiple step static riffle splitter is an appropriate choice for WPC80. This is especially true when sampling for analysis of particle size or functionality, including rheological measurement of powder flowability. Method B is less appropriate method for sampling than A when representative samples is required, from a 20 kg sack of WPC80.

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