Campden BRI
food and drink innovation

Avoiding the pitfalls of nutritional analysis

White paper

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Avoiding the pitfalls of nutritional analysis

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Using emerging ingredients may allow product developers to improve the nutritional profile of products, boosting or adding nutrients and allowing claims to be made. With limited or variable nutritional levels often available from literature it is likely that nutritional analysis will be required. This can prove challenging, as different forms of analysis will yield different results depending on which compounds they detect. In order to aid selection of emerging ingredients to make health and nutritional claims, or simply boost nutritional profile, it is important to ensure the correct approach is used and that there is an awareness of the potential pitfalls. **This briefing note considers these. If this is an area in which you need technical support, please get in touch.**

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General considerations

Ensuring the correct method of analysis is chosen, to capture an accurate nutritional profile, relies on accounting for the variability of several factors. These can include:

- Homogeneity - the product may have an intrinsic variation in makeup, and this is important to understand if tolerances are to be established. This is most common with primary production and raw ingredients, where adjustment for nutrient variation is more challenging.
- Process - the larger the number of samples taken from production the more representative the data set, as nutritional values may vary throughout a run. This must be weighed up against cost of testing and resources.
- Ingredient - it is important to ensure the sampling process takes seasonal and batch variability of ingredients into account.

For nutritional labelling, the Food Information for Consumers (FIC) Regulation states:

> *The declared values shall, according to the individual case, be average values based on:*
>
> *a) the manufacturer's analysis of the food;*
>
> *b) a calculation from the known or actual average values of the ingredient used; or*
>
> *c) a calculation from generally established and accepted data.*

Despite the fact that nutritional analysis can be based on theoretical values, due to the nature of emerging ingredients, there may not be sufficient established data to calculate a profile; this may make it necessary to conduct nutritional analysis.

Understanding the limitations of a method can help when choosing the correct way to detect the desired nutrients. The limit of detection for a method is also an important factor, as it may affect whether you can detect the nutrient in the product. This is particularly important for micronutrients such as vitamins and minerals.

Health and nutrition claims

When making a claim on packaging, pre defined levels of the nutrient must be present in the final product. Whilst it is simple to test the product and establish these levels, it would also be prudent to factor in the level of error associated with the method used. This means that if the result is close to the border of whether a claim can be made or not, the developer can have confidence that it will be above the limit each time it is analysed. In order to make a claim the product has to fall above the threshold at all times, in contrast to the FIC nutritional labelling mentioned above.

Understanding your ingredient

When undertaking analysis of an emerging ingredient it is important to understand which form of each nutrient is expected to be present, to ensure the correct analytical methods are used. This can be achieved by doing literature searches into the ingredients in question, to ascertain the original source, for example from a specific plant in a specific region. This will give an indication of the nutritional profile which may be present. Ingredients suppliers can also help by providing specifications for their products.

Accreditation

To help given confidence in a test method, where possible, it should be ensured the lab has acquired the appropriate level of accreditation. For example, it may be necessary for the method of choice to follow ISO 17025, also known as UKAS accreditation. A UKAS accredited method will give the customer and the lab more confidence in their results and the analyst is required to monitor quality control to ensure the analysis is showing consistency.

FIC nutritional analysis

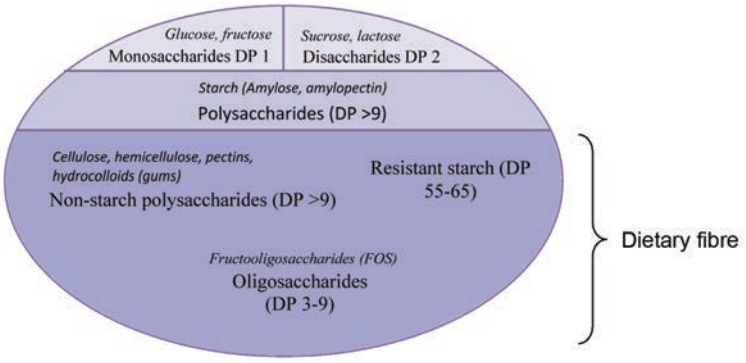
Regulation (EU) No 1169/2011 on the provision of food information to consumers brings together EU rules on general food labelling and nutrition labelling. For nutritional labelling there are several requirements set out within this legislation and it is important for manufactures to be fully aware of these. Ensuring the information given to the consumer is correct requires manufacturers to have knowledge of the nutritional profile of their product, and understanding how to analyse for the different macronutrients will help to ensure nutritional information is accurate and representative. Below are some considerations for different nutrients and how to ensure they are analysed correctly.

Carbohydrates

Classification

Carbohydrates are a major source of energy in the diet and include a range of organic compounds all containing carbon, hydrogen and oxygen. They are based on a common unit with varying chain

Figure 1. The recognised groups of carbohydrates. The individual carbohydrate is defined by the combination of monomer units within these fractions (examples of each type are in italics) (Dai and Chau, 2016)



lengths and linkages. Classification depends on the identity of the individual monomers (such as glucose, arabinose etc.), the degree of polymerisation (DP) and the type of linkage (α or β). This divides carbohydrates into three distinct fractions, see figure 1.

Calculation

When assessing the carbohydrate content of emerging ingredients it is essential that the method takes into account the variance in how these fractions are classified in different countries.

Table 1. How total carbohydrates are calculated in the EU compared to the USA (FAO, 2002)

Total carbohydrates
By difference: *Total carbohydrate = Total weight (g) - (Weight (g) of protein + fat + water + ash + alcohol)* *By direct analysis:* *Total carbohydrate = Weight (g) of mono + di + oligosaccharides + polysaccharides, including fibre**
*Items included in US calculation only, all other items are in calculations both for the EU and US. **All calculations are based on a total weight of 100g**

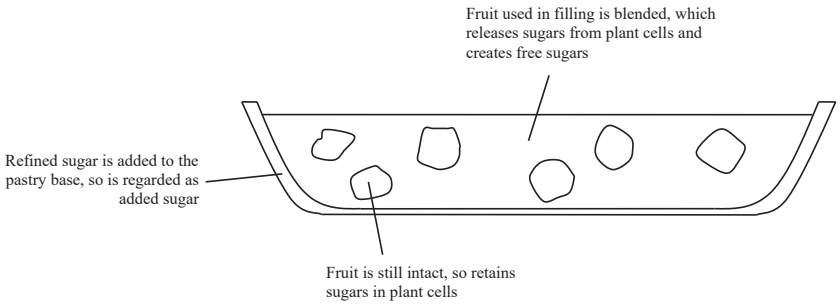
Sugar

Total sugars analysis will typically take into account all mono and disaccharides present in the sample but it is of interest to note that there is guidance (and regulation in some parts of the world) to take into account how sugars are incorporated into products.

UK guidance on ‘free sugars’

In 2015 The Scientific Advisory Committee on Nutrition (SACN) produced guidance that suggested that ‘free sugars’ should account for no more than 5% daily dietary energy intake. The term ‘free sugars’ was adopted, replacing the terms Non Milk Extrinsic Sugars (NMES) and added sugars. Guidance stated that ‘free sugars’ are those added to food or those naturally present in honey, syrups and unsweetened fruit juices, but exclude lactose in milk and milk products (SACN, 2015).

Figure 2. Example of where ‘free sugars’ are likely to be present in a pie product



Whilst the amount of total sugars is displayed on pack information, the levels of free sugars are not shown. In order to understand the difference we need to understand where they came from, whether added as a refined sugar or naturally present in ingredients such as fruits.

It is also important to note that analyse for free sugars is not available, as once you subject a sample to the preparation procedure required for sugars analysis the sugars naturally present, contained within plant cells, are released and in the same form as free sugars. As a result, no added sugar claims stem from the formulation of a product.

US labelling of added sugars

In the US, packaging labels show the amounts of added sugars present in a product. ‘Added sugars’ includes all monosaccharides and disaccharides added to foods. This includes refined sugars, as well as ones present in honey, syrups, nectars etc. They are no longer contained within plant cells; this can be due to blending or pureeing of ingredients. Sugars naturally present in milk products, grains, nuts, seeds are not considered added sugars.

Polyols

Polyols are often mistaken for other types of carbohydrates such as starch. Dietary fibre such as polyols contributes 2.4 kcal/g, compared to starch or sugar carbohydrates which contribute 4 kcal/g. This is because dietary fibre is not absorbed in the small intestine, but some metabolism still occurs in the large intestine and therefore still contributes some energy. If analysts are not aware that a product contains polyols, these will be incorrectly accounted for within the total carbohydrates. As a result, when the calorific content of the food is calculated, the calorie content will be higher than in reality. If polyols are present, a different method of analysis is used to quantify them and, therefore, the energy content of the product can be more accurately calculated.

Dietary fibre

There are several definitions of dietary fibre and this can cause confusion as to which group your ingredient’s components will fall under.

It is clear that there are still variations in the definition of dietary fibre and those fractions included in the definition; therefore, when analysing an emerging ingredient, the method of analysis selected needs to consider the market in which the product is going to be sold in.

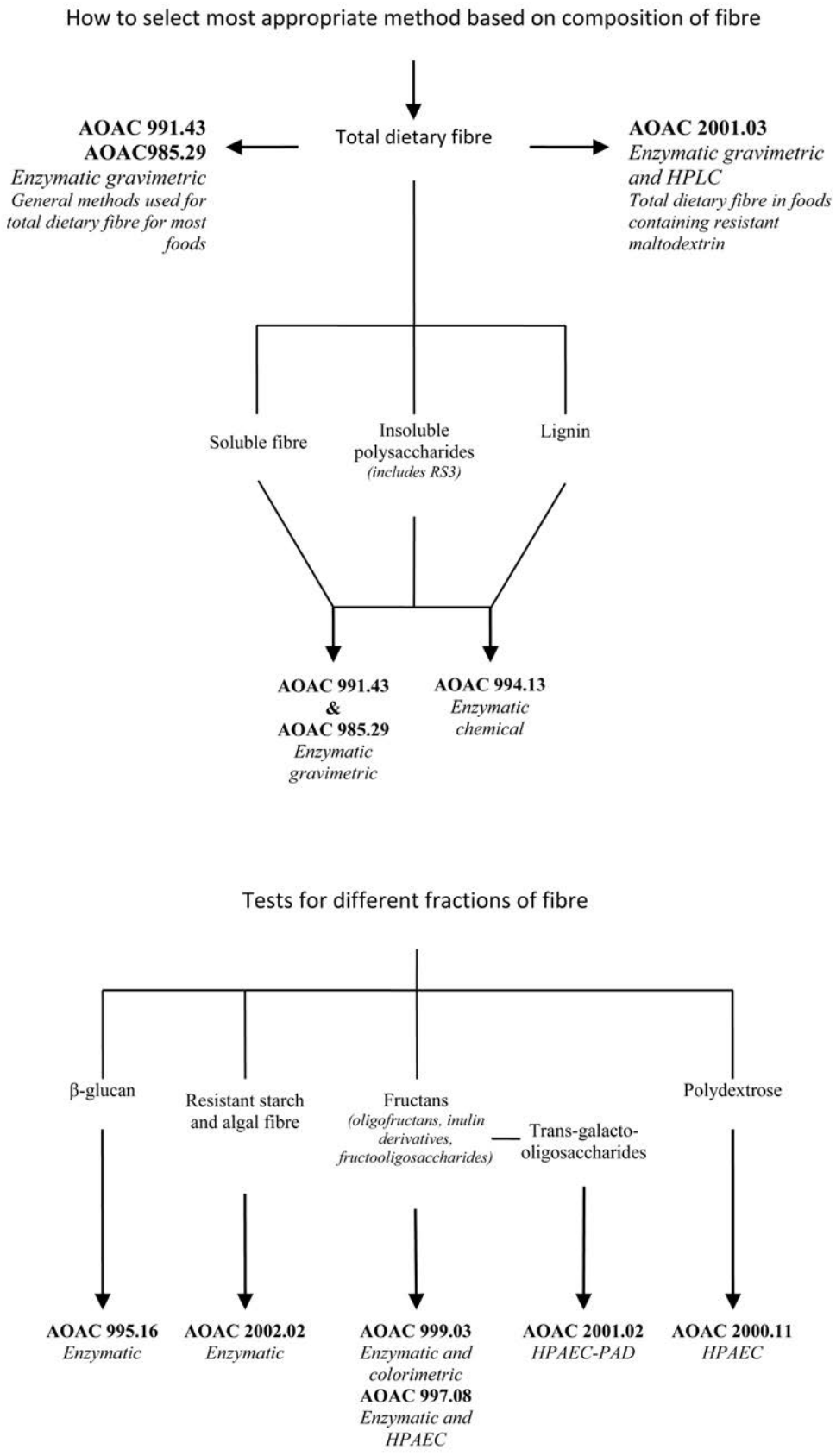
It is also important to understand the difference in what is classified as soluble and insoluble fibres. This is another way of classifying non-starch polysaccharides (dietary fibre) based on their solubility, and the way that it behaves physiologically. Soluble fibres solubilise in water and may have effects on glucose and lipid adsorption from the small intestine, whereas insoluble fibres are those which are slowly and incompletely fermented in the colon and have more pronounced effects on bowel habit. Soluble fibres include pectin and β-glucans while insoluble fibres include cellulose and hemicullulose (SACN, 2015).

Table 2. Definitions of dietary fibre (Zielinski *et al.,* 2013)

Term	Definition	Significance
Non-starch polysaccharides - UK, 1991	Non-α-glucan polysaccharides: cellulose and non-cellulose polysaccharides (e.g. pectins, glucans, arabinogalactans, arabinoxylans, gums, and mucilages)	Only applies to the UK market
Dietary fibre - WHO, 2006	Intrinsic plant cell wall polysaccharides, i.e. non-starch polysaccharides	Not as specific, so would be wiser to focus on a more national definition if possible
Total dietary fibre - US, 2005	Non-digestible carbohydrates and lignin that is intrinsic and intact in plants, and isolated, non-digestible carbohydrate components that have beneficial physiological effects in humans, with a DP of three or more. It was noted that the methodologies used at that time chemically defined total dietary fibre as non-starch polysaccharides, some resistant starches, lignin and some inulin, but did not include non-digestible oligosaccharides	Products from the US are likely to fall into this definition
Dietary fibre - Codex, 2008	Carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by endogenous enzymes in small intestine of human beings plus lignin and/or other compounds when associated with polysaccharides in the plant cell walls. The decision on whether to include carbohydrates from three to nine monomeric units in the definition of dietary fibre was left to national authorities.	Arguably the most detailed and informative description of all of these. Also allows for variation between countries when defining oligosaccharides
Dietary fibre - EFSA, 2010	Non-starch polysaccharides, all resistant starches, all non-digestible oligosaccharides with three or more monomeric units and other non-digestible, but quantitatively minor components that are associated with the dietary fibre polysaccharides, especially lignin.	This is likely to affect products for the EU market

The flow charts (see figure 3) outline the different analytical methods used to measure the different fractions of dietary fibre. Many companies wishing to reduce the calorie content of products and increase fibre are using ingredients such as inulin or fructooligosaccharides (FOS). A key consideration, however, is that the most commonly used methods for dietary fibre do not capture the presence of inulin or FOS, so the total carbohydrate value will appear higher and the dietary fibre value lower than it is in reality.

Figure 3. Flow chart to illustrate fibre analysis options (Zielinski *et al*., 2013)



Protein

The protein content of foods is calculated via total nitrogen content; the nitrogen content is then multiplied by a factor to determine protein content. This approach is based on two assumptions:

- That dietary carbohydrates and fats do not contain nitrogen
- That nearly all of the nitrogen in the diet is present as amino acids in proteins

On this basis, as the average nitrogen (N) content of proteins is about 16%, a conversion factor of 6.25 is used. However, not all nitrogen in foods is found in proteins; it is also contained in variable quantities in a variety of organic compounds such as melamine or ammonia, where it is referred to as non-protein nitrogen (NPN).

Furthermore, the nitrogen content of specific amino acids can vary according to the number of nitrogen atoms it contains. Due to this the nitrogen content of proteins actually varies from about 13% to 19%; as a result a range of factors are required to calculate protein content across different food groups. **NB: this is not usually chosen by the lab, which will use the standard 6.25 unless the client specifically requests another factor.**

Table 3. Protein factors for different foods (Finglas *et al.,* 2015)

Ingredient	Factor
Wheat wholemeal flour	5.83
Wheat flours (not wholemeal)	5.70
Wheat pasta	5.70
Wheat bran	6.31
Maize	6.25
Rice	5.95
Barley, oats, rye	5.83

Ingredient	Factor
Soya	5.71
Peanuts, brazil nuts	5.46
Almonds	5.18
All other nuts	5.30
Milk and milk products	6.38
Gelatine	5.55

For foods with more than one ingredient a factor of 6.25 is used, unless it has been specifically requested that a different factor is used. For foods that contain a measureable amount of non-protein nitrogen, this amount should be subtracted before multiplication by the appropriate factor. As before, this will only be done by request, and the NPN of foods is usually negligible (Finglas *et al*. 2015).

REGULATION (EU) No 1169/2011 defines that the protein content is calculated using the formula:

protein = total Kjeldahl nitrogen × 6.25

This is important to note when getting nutritional analysis completed for back of pack nutritional analysis, to ensure the appropriate factor is used. It is also important to note that the method of analysis used will affect what NPN is picked up, for example, the Kjeldahl method measures ammonia forms of nitrogen so non-protein sources of ammonia will be picked up as well as melamine, whereas, the Dumas method pick up all forms of nitrogen.

Vitamins

There are several forms of the same vitamins which can be present in a variety of products. For example, vitamin A can be expressed as retinol in animal sources, or carotenoids in plants. Therefore, when requesting analysis for a vitamin in an emerging ingredient, it is important to consider the source, and discuss this with the analyst to ensure the correct form is analysed for.

Many vitamins are present as several isomers (compounds with the same chemical formula but different structure) so when requesting analysis it is often necessary to consider whether the total of an exact isomer or total amount of all the forms of the vitamin are required. Often, not all isomers are biologically active. When asking for an analytical result to be reported it is important to identify which form the result needs to be reported in to ensure the correct conversion factors are used.

There are an increasing variety of analytical methods which are designed to improve the extraction of the different forms of vitamins, but this may lead to a lack of consistency between labs in detected levels, as some may have increased extraction rates compared to others. Consequently, for continual monitoring of vitamins, it may be wise to analyse samples with the same lab to prevent variation in results. If a developer is unsure whether the results are valid it may be prudent to have the same samples tested in another laboratory to validate these levels.

It is also important to identify whether the vitamin is from a natural source or fortified, as this affects the extraction method used. If the vitamin is naturally present within the matrix of the product it will need a different extraction method to a dried powder which has been dissolved.

Several vitamins are sensitive to environmental factors such as light and oxygen. Therefore, when preparing the same to be sent for analysis, the sample should be sent frozen in a sealed container which will not allow light to pass through to minimise losses.

The matrix of a product can affect analysis of certain vitamins; therefore it is important to consult the analyst to identify whether any preparation of the material being testing is required. An example of this is when analysing for vitamin D, where moisture content plays a large factor in the recovery from the sample (table 4).

Minerals and metals

Analysis for salt can be expressed in terms of total sodium or chloride, which could result in other compounds influencing the total salt content. This is because another compound may contain sodium or chloride ions, this is of particular importance in low salt products which may use potassium chloride as a salt replacer. If there are other components with sodium or chloride present they may cause a high salt result unintentionally.

Understanding whether the nutrient is naturally present or present through fortification will help to anticipate the expected levels from which the method can be adapted appropriately.

Table 4. Examples of forms for vitamins

Vitamin	Forms	Additional comments
Vitamin A (fat soluble)	Retinol - natural form but can be added synthetically. Retinyl palmitate and retinyl acetate - synthetic form	• From animal sources • In general all forms are converted to one form during analysis and then quantified. For quantification of one form, must specify when ordering analysis to allow use of correct conversion factors
	Carotene (alpha and beta) - natural form	• From plant based sources • The body converts beta-carotene to vitamin A • Not typically added synthetically due to how unstable it is • If given the result as carotenes - need to convert to retinol equivalents
Vitamin B (water soluble)	B1; Thiamin - natural form. Thiamine mononitrate and thiamine hydrochloride - synthetic form	• Usually reported as thiamine hydrochloride due to analytical method
	B2; Riboflavin - natural form. Riboflavin monophosphate - synthetic form.	
	B3; Niacin - sum of nicotinic acid and niacinamide	• Sometimes tryptophan is also measured, as thought to contribute to making B3 in the body, but no way to determine how much
	B5; Pantothenic acid - natural form. Sodium Pantothenic acid - synthetic form	
	B6; Three isomers - pyridoxine, pyridoxamine, and pyridoxal - natural form Pyridoxine hydrochloride - synthetic form	• Usually reported as pyridoxine hydrochloride due to analytical method • Pyridoxamine, and pyridoxal not normally added synthetically • Different methods can be used to distinguish different isomers
	B12; Cobalamin - natural form with different “cores” e.g. H, OH, Adenyl, H_2O Cyanocobalamin which is a form which exists in nature and is the most stable of the cobalamins. This form is most used to fortified foods.	
	B9; Folates - natural form Folic acid - synthetic form	
	Biotin	• Same form whether from a natural or synthetic source
Vitamin C (water soluble)	Ascorbic acid (L-ascorbic acid) - natural form	• Multiple synthetic forms available (usually the salt form of the acid)

Table 4. Examples of forms for vitamins (continued)

Vitamin	Forms	Additional comments
Vitamin D (fat soluble)	D2 (Ergocalciferol)	• No difference between form from natural or synthetic sources • Sometimes added to a sugar unit to encapsulate the vitamin making it more stable, therefore a different extraction method has to be used • Natural source is from plants and fungi
	D3 (Cholecalciferol)	• No difference between form from natural or synthetic source • Sometimes added to a sugar unit to encapsulate the vitamin making it more stable, therefore a different extraction method has to be used • Natural source is from animals
Vitamin E (fat soluble)	Alpha-Tocopherol (DL - alpha tocophenyl acetate as conversion from alpha tocophenol) DL - alpha tocophenol D - alpha tocophenol	• Four forms present naturally; alpha, gamma, beta and delta • The different forms have different levels of bioactivity (gamma and delta forms have 10% the bioactivity alpha). • When making a claim, if naturally present quantify all forms present, however when adding synthetically only add alpha form • For quantification of one form, must specify when ordering analysis to allow use of correct conversion factors
Vitamin K	K1 (Phylloquinone)	• More forms however K1 and K2 and only forms found in food consumed by humans • K3 is often added to pet food but is toxic to humans
	K2 (Menaquinone) MK4 - natural form but can be added synthetically MK7- synthetic form	

Bioavailability of nutrients

It is important to consider, with new and emerging ingredients, that their nutritional profile is not necessarily linked with the bioavailability of the nutrients within them. As emerging ingredients are not as widely used or explored, it may mean that the nutritional availability of their carbohydrates, proteins etc. may not be as effective or the same as other foods.

Conclusion and next steps

Whilst there is a wealth of emerging materials in which we can find new ingredients, extra care should be taken when looking to understand their nutritional impact on the diet of the consumer. Having a more targeted view of how the ingredient will be consumed, where it will be sold, and the components

of the product will help developers to create a product which they have complete confidence in. It will also help to pick the right method of analysis to get a representative nutritional profile.

At Campden BRI we have an extensive range of nutritional analysis capabilities and can advise on analysis methods and how to interpret analysis results. If you would like to find out more about this area or if you need technical support please get in touch.

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About Campden BRI

Campden BRI helps food and drink businesses succeed

We do this through practical scientific, technical and knowledge support

We work closely with industry to ensure the absolute relevance of all our activities - from analysis and testing, process validation and safety assurance to product innovation, consumer studies and training, events, databases and publications

All our activities are underpinned by a strong programme of research - steered by industry for maximum relevance

Membership-based, we provide services to companies all along the supply chain

Vision

To be the partner of choice for the development and application of technical knowledge and commercially relevant solutions for the food and drink chain

Mission

Practical application of technical excellence for the food and drink chain

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