# Jungbunzlauer

From nature to ingredients.

# facts

Calcium and zinc fortification of infant and child products



#### Introduction

Minerals are indispensable nutrients in infant and child nutrition. According to Innova Market Insights for 2020 to 2022, brain health, immune and bone health are all in the top four health claims for product launches in the "baby and toddler" category. These are very relevant claims for child health, which are best addressed via calcium and zinc fortification. But how can this be achieved in a safe and sustainable way while complying with the strict nutritional requirements and regulations in this sensitive food segment, and how can it be achieved in the related child supplements category?

Jungbunzlauer is an important manufacturer of value-adding ingredients produced by the natural process of fermentation. This ties into our sustainability record and ambitions, which we publish in our annual sustainability report.

This technical paper, which focuses on infant and child products, compiles regulatory and bioavailability information to serve as a starting point for selecting appropriate calcium and zinc salts, followed by a detailed description of successful and tasty formulations for various products, ranging from complementary food, to drinks and snacks, to supplements.

# Regulatory environment

In Europe, minerals must not be added to infant and baby foods unless they are listed in Regulation 609/2013 for the relevant baby or infant category. A similar list can be found in Codex Guideline CXG 10-1979.

In the US, the use of mineral salts is not clearly regulated. For infant food, only products listed by the Food and Drug Administration (FDA) as "Generally Recognized as Safe" (GRAS) should be used. Use of mineral salts in older children's categories, however, is up to the food producer. Mineral salts with self-affirmed GRAS status may also be used.

Table 1 gives an overview of the approvals of calcium and zinc salts of Jungbunzlauer, which were used to fortify the products described in this paper.

Table 1: Approvals of Jungbunzlauer products

Mineral	EU*	Codex**	US
Tricalcium Citrate	<b>✓</b>	<b>~</b>	FDA GRAS 21 CFR §184.1195
Zinc Citrate	<b>✓</b>	<b>✓</b>	Self-affirmed GRAS
Zinc Gluconate	<b>~</b>	<b>✓</b>	FDA GRAS 21 CFR §182.8988

<sup>\*</sup> Listed in Annex I of Regulation 609/2013/EC

<sup>\*\*</sup> Listed in CXG 10-1979

# Calcium and zinc salts: health claims and bioavailability

Calcium, one of the five most important minerals in the human body, is particularly important for the healthy development of children. The importance of calcium for child development has been evaluated by the European Food Safety Authority (EFSA), which confirmed two health claims for calcium fortified products for children.<sup>[1, 2]</sup>

The following health claims are therefore permitted under Article 14(1)(b) of Regulation (EC) 1924/2006:

- Calcium is needed for normal growth and development of bone in children.
- Calcium and vitamin D are needed for normal growth and development of children's bones.

Zinc is an essential trace element that plays a crucial role in the function of more than 200 enzymes involved in major metabolic pathways and cell division. Zinc deficiency is common among children in many developing countries and leads, among others, to growth retardation and impaired immune function. In its scientific opinion, the EFSA also identified a link between dietary intake of zinc and the normal function of the immune system, and therefore a resulting beneficial physiological effects of zinc on infants and young children. However, this health claim is not mentioned in Regulation (EC) 983/2008 on the authorisation of health claims referring to reduction of disease risk and to children's development and health. Therefore, only nutritional claims related to zinc are currently permitted in the EU.

Reference values for the EU and the US are listed in table 2. In the EU, nutrient labelling must comply with the Food Information Regulation. <sup>[4]</sup> In the US, nutrition and health claims are permitted as outlined in the FDA's Code of Federal Regulations, Title 21 (21 CFR), Part 101. Reference values are outlined in 21 CFR §101.9(c)(8)(iv). Corresponding nutrient content claims are allowed in accordance with 21 CFR §101.54.

Table 2: Calcium and zinc nutrient reference values (NRVs)

Mineral	EU, children under 3 years <sup>[5]</sup>	EU, children 3 years and over <sup>[4]</sup>	US, children 1–3 years	US, adults and children 4 years and over
Calcium	400 mg	800 mg	700 mg	1.3 g
Zinc	4 mg	10 mg	3 mg	11 mg

The organic mineral salts used in the projects described in this paper are salts resulting from neutralisation of naturally occurring acids – such as citric or gluconic acid – by a source of calcium or zinc. These salts usually have a more neutral taste than inorganic mineral salts and, in some cases, can even reduce off-notes, such as bitterness in dairy alternative products.<sup>[6, 7]</sup>

The nutritional effectiveness of minerals is highly dependent on their bioavailability, which is the ability of the body to absorb the minerals and make them available for use. It is known from numerous studies that bioavailability can vary greatly depending on the source. For example, calcium absorption can vary between 5% and 40% depending on which mineral salt is used. For zinc, this variation can be as great as 5% to 80%. In most previous studies, organic mineral salts demonstrated higher bioavailability than inorganic salts<sup>[8-11]</sup>, which is a highly important factor for meeting the nutritional needs of babies and young children during their physical and cognitive development.

# **Sensory evaluation**

Sensory evaluation is a scientific method used to measure, analyse and interpret responses to products as perceived through human senses. Properties of a product such as flavour, taste, appearance and texture can be determined and described through human sight, hearing, smell, taste and touch. At Jungbunzlauer, tests are conducted in a climate-controlled, odour-free and quiet testing area with separated booths. The internal taste panel at Jungbunzlauer consists of approximately 35 continuously trained panellists.



# **Triangle test**

A triangle test is a common test method used to find out whether two products or recipes differ significantly from one another. The panellists are given three samples, of which two are the same and one is different; for example, a fruit bar without any additional components might be compared to a fruit bar with added mineral salts. The panellists taste the samples in the order they are presented and must decide which one of the three samples is different from the other two. The test is conducted using blind labelling (three-digit codes), and the order in which the samples are presented is randomised for each panellist.



# Mineral fortified fruit puree

Fruit puree products such as fruit squeezes are very popular among children. Fortification with minerals can add extra value to these products. Various calcium salts are available for calcium fortification. In this project, organic tricalcium citrate was compared to two inorganic mineral salts: tricalcium phosphate and calcium carbonate.



## Results

The effect of different calcium salts on the pH of plain fruit puree (pH 3.7) was investigated by adding them at a concentration of 120 mg calcium per 100 g puree (15% NRV), which is necessary for a calcium claim for children of age 3 and older (EU). This resulted in a slight pH increase to 4.0 with calcium citrate, a moderate increase to 4.3 with tricalcium phosphate and a large increase to 5.1 with calcium carbonate.

The extreme effect of calcium carbonate on pH not only negatively affects shelf life, but also has a strong impact on taste. For this reason and due to the risk of gas formation in the acidic pH range, calcium carbonate in these quantities is not suitable for this application without adjusting the recipe by adding extra acid.

A triangle test comparing tricalcium citrate and calcium carbonate revealed a highly significant difference. The puree with tricalcium citrate was described as fruity, refreshing, sour, and less sweet, and thus comparable to the plain puree.

In two further sensory tests, the plain puree was tested against a version with tricalcium citrate and a version with tricalcium phosphate. The calcium concentration was again 120 mg per 100 g of puree. No significant difference was found between the plain puree and the version with tricalcium citrate.

However, when tricalcium phosphate was compared to the plain puree, a significant difference was found. The addition of tricalcium phosphate resulted in the puree being perceived as flatter in taste, less fruity, milder and sweeter.

No changes were observed in the fortified product over a storage period of three months. Similarly, a rheological measurement showed that use of tricalcium citrate had not caused any change in viscosity compared to the unfortified product (fig. 1).

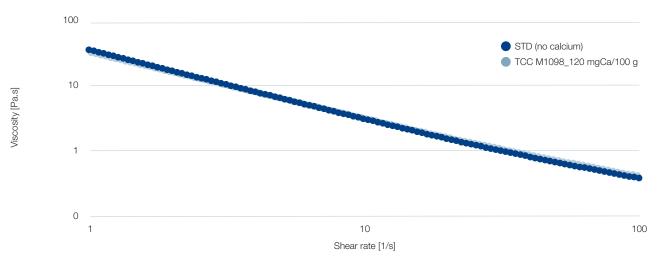


Figure 1: Viscosity measurement of mineral fortified fruit puree

# **Summary**

Regarding the three salts tested, it can be concluded that calcium enrichment without affecting flavour can only be achieved with tricalcium citrate. Moreover, tricalcium citrate caused less of a rise in pH than inorganic calcium salts. Therefore, overall dosage can be higher without altering product properties when tricalcium citrate is used.

Adding 120 mg calcium per 100 g is sufficient for a product for children older than three years to be declared a "source of calcium" in the EU. In the US, addition of these quantities is sufficient for a product (125 g serving size) for toddlers aged one to three years to be declared "high in calcium".

Table 3: Calcium fortified fruit puree recipe

	Ingredients	Supplier	Quantity		
1	Apple puree		59.66%	60.00 g	
2	Banana puree		19.89%	20.00 g	
3	Strawberry puree		19.89%	20.00 g	
4	Tricalcium Citrate M1098	Jungbunzlauer	0.57% 0.57 g		
	Total		100%	100.57 g	

#### Mineral fortified fruit bar

Healthy snacks should be within every parent's reach, and fruit bars are a very popular product for children. We at Jungbunzlauer aim to add the extra benefit of mineral fortification to these products, which may already be a good source of vitamins such as ascorbic acid. For this test, a recipe for a mineral fortified fruit bar was developed and analysed in terms of flavour, mouthfeel and technological properties such as  $CO_2$  development as well as pH stability during and after the production process.

A challenge in this project was the question of whether it was possible to add calcium and zinc without creating any negative impacts on appearance, flavour or mouthfeel of the fruit bar.



#### Results

Initial trials showed that unlike calcium carbonate, tricalcium citrate does not lead to formation of  $CO_2$  in an acidic environment. The texture of such products containing tricalcium citrate therefore remains stable. Triangle testing was conducted to assess the sensory properties of the fruit bars. A comparison of a fruit bar containing Jungbunzlauer tricalcium citrate and zinc citrate to a fruit bar without any additional minerals showed that there was no significant difference between the two samples.

A comparison of a fruit bar containing tricalcium citrate to one containing tricalcium phosphate revealed no significant difference. With regard to the sustainability challenges around phosphates<sup>[12]</sup>, replacing them with alternatives is an important topic for food manufacturers. Another study conducted by Jungbunzlauer also revealed that organic mineral salts are healthier and more natural; and, in a direct comparison, citrates were preferred over phosphates.<sup>[13]</sup>

A comparison of a fruit bar containing tricalcium citrate to one containing calcium carbonate showed that there was a significant difference between the two samples. The fruit bar containing tricalcium citrate was described by the panellists as fruitier, more flavourful, refreshing and having a nicer texture than the fruit bar containing calcium carbonate. The latter was described as less fruity, with a flatter taste, an unpleasant aftertaste and a softer and floury/chalky texture.

#### **Summary**

Tricalcium citrate, zinc citrate and zinc gluconate can be used for mineral fortification without compromise in appearance, flavour or texture. We were able to demonstrate advantages of using Jungbunzlauer tricalcium citrate over calcium carbonate – both from a technological and a sensory perspective. In contrast to calcium carbonate, tricalcium citrate does not lead to formation of CO<sub>2</sub> in an acidic environment, has less of an impact on pH, tastes better and has a more pleasant mouthfeel. It was also found that phosphates could be replaced by citrates without compromise in taste or texture. Addition of Jungbunzlauer Special Salts enabled us to achieve a sufficiently high mineral content to claim "source of calcium" and "high in zinc" for the EU market, and "high in calcium" and "high in zinc" for the US market.

Table 4: Mineral fortified fruit bar recipe

	Ingredients	Supplier	Quantity	
1	Banana flakes		39.00%	8.97 g
2	Apple juice concentrate (70°Bx)	Th. Geyer	30.00%	6.90 g
3	Whole oat flour		17.36%	3.99 g
4	Sunflower oil		5.00%	1.15 g
5	Raspberry juice concentrate (65°Bx)	Th. Geyer	3.00%	0.69 g
6	Tricalcium Citrate M1098	Jungbunzlauer	2.89%	0.66 g
7	Edible paper (with potato starch)		2.20%	0.51 g
8	Acerola juice concentrate (65°Bx)	Th. Geyer	0.55%	0.13 g
9	Zinc Gluconate	Jungbunzlauer	0.015%	0.0035 g
	Total		100%	23 g

#### Zinc fortified children's biscuits

Biscuits are very popular among children. Accordingly, a huge variety of products is available on the market to meet their specific needs. Nevertheless, children's biscuits fortified with minerals are still rare. Zinc is an essential trace element, supporting many processes in the body including a normal function of the immune system. The COVID-19 pandemic has led to increased awareness among parents regarding the health benefits of the snacks they offer their children. To address this trend, Jungbunzlauer developed a children's biscuit with an additional health benefit by including zinc citrate in the recipe.

#### Results

A standard biscuit recipe (table 5) was used to test the effect of zinc fortification on biscuits.

Table 5: Zinc fortified biscuit recipe

	Ingredients	Supplier	Quantity		Baker's percentage
1	Whole wheat flour	Lantmännen	32.05%	128.20 g	
2	Corn flour		13.75%	55.00 g	100%
3	Oat flour		13.75%	55.00 g	
4	Sugar		14.32%	57.30 g	24.06%
5	Potassium bicarbonate		0.85%	3.40 g	1.43%
6	Glucono-delta-Lactone F2500	Jungbunzlauer	1.02%	4.10 g	1.72%
7	Zinc Citrate Trihydrate	Jungbunzlauer	0.015%	0.06 g	0.025%
8	Vegetable oil		12.35%	49.40 g	20.74%
9	Water		11.90%	47.60 g	19.98%
	Total		100%	400 g	

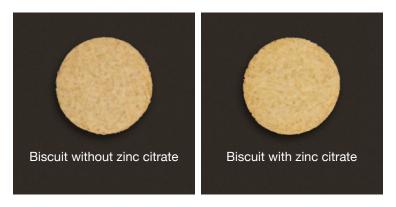
EU: addition of 0.46 mg thiamin/100 g biscuits is required by Directive 2006/125/EC.

Preparation of the biscuits started with sieving three types of flour, glucono-delta-lactone and zinc citrate and measuring them into a bowl. The reference biscuits were prepared without zinc citrate. The dry blend was then mixed for 1 min. Water and potassium bicarbonate were weighed into a separate bowl and mixed for 5 min. After the potassium bicarbonate was properly dissolved, oil and sugar were added and the slurry was mixed for 3 min. The dry blend was then added to the slurry and the resulting dough was kneaded for precisely 1 min. After 15 min of resting time at room temperature, the dough was rolled out and 20 biscuits were portioned with a biscuit cutter (4.1 cm diameter) within 10 min. The biscuits were then baked for 15 min at 180°C (356°F) with top and bottom heat.

The biscuits were visually inspected one day after baking. The height and diameter of every biscuit were measured with a calliper, and the spread of each biscuit was calculated by dividing diameter by height. The hardness of the biscuits was determined one day after baking and again after 4 weeks of storage by conducting a three-point bending test with a Stable Micro Systems TA.XTplusC Texture Analyser. For sensory evaluation, a triangle test was conducted in order to determine whether significant differences in taste and texture could be identified between biscuits fortified with Jungbunzlauer zinc citrate and unfortified biscuits.

The visual inspection revealed no detectable difference in appearance (colour, shape, surface) between fortified and unfortified biscuits (figure 2).

Figure 2: Unfortified (left) and zinc fortified (right) children's biscuits



Addition of zinc also had no negative effect on the spread of the biscuits (n=20): unfortified biscuits had a spread of 6.0, while the fortified biscuits had a spread of 5.9. Regarding hardness, the fortified biscuits (n=5) were slightly harder (+1 Newton) than the unfortified biscuits (n=5). However, this difference in hardness was not detected in the triangle test (n=11). Flavour was also clearly not influenced by the addition of zinc citrate: the panellists were unable to distinguish between fortified and unfortified biscuits.

#### **Summary**

Overall, the addition of zinc citrate did not affect the appearance, spread, hardness or flavour of the biscuits. Therefore, zinc citrate can be added to biscuit recipes to provide additional health benefits without influencing taste, texture or appearance.



#### Fortified oat drinks for children

Among the great variety of milk alternatives, oat drinks are particularly popular. Oats are a naturally gluten-free cereal which can be grown in many regions of the world, offering a well-balanced amino acid profile and good sustainability. These factors make oats an attractive ingredient for milk alternatives.<sup>[14]</sup>

The goal of this project was to develop two mineral fortified cocoa oat drinks, tailored to the EU and US markets, to meet the particular needs of growing children. The project also compared organic tricalcium citrate to inorganic calcium carbonate and tricalcium phosphate in terms of flavour and mouthfeel.

#### Results

To investigate the influence of different calcium sources on pH, taste and mouthfeel of the oat drink, the EU recipe was prepared with tricalcium citrate, calcium carbonate and tricalcium phosphate (calcium content: 120 mg per 100 ml  $\triangleq$  15% NRV). Appearance and storage stability were the same for all three drinks. Addition of tricalcium citrate to the oat drink, which also contained cocoa and banana puree (pH 6.0), implied only a minor increase in pH to 6.6. In contrast, tricalcium phosphate and calcium carbonate resulted in larger pH increases to 7.1 and 7.7, respectively.

In a triangle test comparing the oat drinks with tricalcium citrate and tricalcium phosphate, the panellists were unable to detect any significant difference between the two samples. Organic tricalcium citrate is therefore a viable replacement for inorganic tricalcium phosphate which does not affect any sensory attributes of the product. Phosphates face significant sustainability challenges<sup>[12]</sup>, and replacing them is an important topic for food manufacturers. In a study conducted by Jungbunzlauer, organic mineral salts were also evaluated as healthier and more natural, and citrates were even preferred over phosphates when directly compared.<sup>[13]</sup>

When oat drinks with tricalcium citrate and calcium carbonate were compared to each other in a triangle test, panellists were able to detect a significant difference. The drink containing calcium carbonate was described as sweeter and milder in taste, which may have been due to its higher pH. The oat drink with tricalcium citrate was described as having a more banana-like flavour and slight bitterness (cocoa). However, the influence of tricalcium citrate on the taste of the oat drink seemed to be minimal, as the other ingredients (banana puree, cocoa) came through more strongly. Meanwhile, calcium carbonate seemed to flatten the taste profile.

#### **Summary**

Tricalcium citrate is a good choice when it comes to fortification of oat drinks: it has less of an effect on the pH and taste of the product than it is the case with inorganic calcium sources. To ensure proper suspension of tricalcium citrate, it is advisable to use TayaGel® HA.



# **Immune boost syrup**

The growing trend in immune health in children's supplements inspired the development of an immune boost syrup for children containing vitamin C and zinc. Paired with an increasing demand for products with no added sugar, the syrup used a combination of erythritol and stevia to allow it to be declared sugar-free. In addition, erythritol is non-cariogenic and has a positive effect on dental health.

#### Results

Currently, glycerol is the most commonly used polyol in these types of supplements. Glycerol is a carbohydrate with a high calorie content of 4.3 calories per gram, and can make a large contribution to the final calorie count. Erythritol, such as ERYLITE® from Jungbunzlauer, is a good alternative to vegetable glycerine and sorbitol in sugar-free syrups: it is tolerated well by the digestive system and contains 0 calories per gram. A combination of ERYLITE® and steviol glycoside (Rebaudioside A) creates a pleasant sweetness profile with no lingering off-notes.

US regulations limit the proportion of erythritol in syrups to 15%. Although no such limits exist for syrups in EU, it is important to use the correct erythritol-to-water ratio to avoid recrystallisation: the proportion of erythritol in the total water in the formulation should not exceed 20%. Although the syrup prepared in this project contains botanicals that are associated with immune health, regulations do not allow a claim of "immune-boosting" for these substances. This is where fortification with zinc and vitamin C comes into play.



Zinc gluconate contains 12.5%–14.6% zinc. Its high bioavailability and excellent solubility make it an ideal ingredient for this application. Zinc gluconate was used in the formulation at 0.16%, providing 2.4 mg of zinc per 10 ml serving. The source of vitamin C was the acerola extract in the blend of botanicals, providing 99 mg vitamin C per 10 ml serving. Use of a naturally occurring source of vitamin C fits with the clean label trend. However, this increased the quantity of insoluble botanicals in the formulation.

These particles needed to be suspended for better visual appeal and more consistent distribution of nutrients. The addition of 0.17% of Xanthan gum successfully prevented sedimentation of the insoluble botanical particles. The increased viscosity provided a syrupy consistency and improved the mouthfeel. Tripotassium citrate was used at 0.4% to increase the starting pH of the syrup from 3.4 to 4.0, reducing astringency and improving the overall taste profile.

A twelve-week stability test of this sugar-free recipe revealed no changes to pH, sedimentation of botanicals or recrystallisation of erythritol. A nutritional analysis confirmed that inclusion of vitamin C from acerola extract and fortification with zinc gluconate were sufficient for the syrup to be declared "immune-boosting".

#### **Summary**

A sugar free immune boost syrup for children was developed with erythritol and stevia allowing for a sugar free claim and reduced calories. Erythritol is a good alternative to other commonly used polyols as such products can contain high calories. The syrup is fortified with zinc gluconate and is a source of vitamin C which permits an immunity claim. Xanthan gum has successfully prevented the sedimentation of insoluble botanicals.

#### Conclusion

Overall, the projects considered in this paper have shown that fortification with tricalcium citrate, zinc citrate and zinc gluconate offers better product flavour and processing than with inorganic analogues, while also avoiding negative effects on texture and stability.

In fruit puree, the use of tricalcium citrate resulted in a smaller increase in pH, and therefore better product stability, than with inorganic calcium salts, allowing higher dosages and potentially allowing nutritional claims to be made regarding the product. In fruit bars, tricalcium citrate, zinc citrate and zinc gluconate are all possible minerals which do not influence taste, texture or stability. The same benefits were observed when enriching biscuits with zinc citrate. For oat drink fortification, we recommend tricalcium citrate combined with TayaGel to ensure sufficient suspension. Finally, a sugar-free immune boost syrup with zinc and vitamin C was successfully developed.

The good bioavailability of the organic calcium and zinc salts used ensures effective nutrition, and thus supports the physical and cognitive development of children. For all products, nutrition claims would be permitted in Europe and the US with the amounts used; for the calcium-enriched products, health claims regarding normal growth and bone development would be permitted in Europe.

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# About Jungbunzlauer

Jungbunzlauer is one of the world's leading producers of biodegradable ingredients of natural origin. We enable our customers to manufacture healthier, safer, tastier and more sustainable products. Due to continuous investments, state-of-the-art manufacturing processes and comprehensive quality management, we are able to assure outstanding product quality.

Our mission "From nature to ingredients®" commits us to the protection of people and their environment.

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