# **Research Article**

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## Chemical composition of infant milk formulas sold in Alexandria, Egypt

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## ABSTRACT

**Background:** Infant formula is a synthetic version of mothers' milk belongs to a class of materials known as dairy substitutes. Although the World Health Organization recommends breastfeeding as the best feeding choice, infant formulas remain an alternative to breast-milk and plays an important role in infant's diet. The composition of most infant formulas has been increasingly altered to create a product which attempts to be as similar to breast milk as possible. **Objective:** to analyze the chemical composition of some popular brands of infant formulas sold in Alexandria, Egypt and to compare to the results with those declared by the producers. Materials and Methods: Fifteen formulas were collected from three different types (ordinary, lactose free, and extra care formulas) and analyzed for their proximate principles, mineral and vitamin contents. Results: The highest proximate principles contents were in ordinary formulas except for moisture content where lactose free formula had the highest percentage  $(2.55\pm0.02)$ . The actual protein and fat contents were lower than that declared on the label in all formulas except in ordinary formula (brand 2) and lactose free formula in their protein contents. The highest mineral contents were in ordinary formulas specially (brands 1 and 2), except for iron where lactose free formula had the highest contents ( $6.35\pm0.04$  ppm). The highest contents of vitamins A, D and E were in ordinary formulas (brand 1), ordinary formulas (brand 2) and extra care formulas (587.0  $\mu$ g, 10.68 mg and 15.2  $\mu$ g/100 g; respectively), moreover, the actual vitamin contents were lower than that declared by the manufacturer in all formulas except for ordinary formulas (brand 1) in their vitamin A and D, ordinary formulas (brand 2) in their vitamin D and E contents and except for extra care in their vitamin E contents. Conclusion: There were discrepancies between the actual chemical composition of infant formulas and those declared by the manufactures on their labels. Further studies are required to evaluate the chemical composition of infant formulas on a greater number of samples and a wider diversity of brands to ensure the accuracy of the contents declared on their label.

#### Keywords: Infant Milk Formulas, Proximate Principles, Vitamins, Minerals, Egypt

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## INTRODUCTION

Infant formula is a synthetic version of mothers' milk belongs to a class of materials known as dairy substitutes which are made by blending fats, proteins, and carbohydrates (1). Although the World Health Organization recommends breastfeeding as the best feeding choice, infant formulas remain an alternative to breast-milk and plays an important role in infant's diet (2). When breast feeding is not possible, desirable, or sufficient; infant formulas are often used as substitutes for human milk and play an indispensable role in infant nutrition (3). Besides breast milk, infant milk formulas are the only other milk products which the medical community considers them nutritionally acceptable for infants under the age of one year (4). The composition of infant formulas is based on a mother's milk at approximately one to three months postpartum (5). The most commonly used infant formulas contain purified cow's milk, whey and casein as a protein source, a blend of vegetable oils as a fat source, lactose as a carbohydrate source, a vitamin-mineral mix, and other ingredients depending on the manufacturer. Some infant formulas use Soya bean as a protein source in place of cow's milk and others use protein reduced (hydrolyzed) for infants who are allergic to other proteins (6).

The composition of most infant formulas has been increasingly altered to create a product which attempts to be as similar to breast milk as possible. Many infant milks use comments on their packaging such as "inspired by breast milk" or "as close as possible to breast milk" and suggest that there is 'science' behind their products which makes them very close to breast milk in composition (7). Although cow's milk is the basis of almost all infant formula, plain cow's milk is unsuited for infants because of its high casein content and low whey content which may put a strain on an infant's immature kidneys, and untreated cow's milk is not recommended before the age of 12 months (5).

The infant intestine is not properly equipped to digest non-human milk and this may often result in diarrhea, intestinal bleeding and malnutrition. Therefore cow's milk used for formulas undergoes steps in order to make protein more easily digestible and alter the whey-to-casein protein balance to a ratio closer to human milk, addition of essential ingredients "fortification", and partial or total replacement of dairy fat with fats of vegetable or marine origin (6). The aim of the present study was to analyze the chemical composition of some popular brands of infant formulas sold in Alexandria, Egypt and to compare to the results with those declared by the producers.

## MATERIALS AND METHODS

A total of fifteen infant milk formulas were collected from three different types sold in pharmacies of Alexandria, Egypt (ordinary, lactose free, and extra care formulas). The ordinary formulas were collected from three different commercial brands. Three samples were collected from the lactose free formulas, extra care formulas, and from each of three brands of the ordinary formulas. All samples were powder, cow milk based formulas packaged in tin cans.

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### Moisture content

A sample of five-grams sample was dried in the oven at  $102^{\circ}C \pm 2^{\circ}C$  for 2 hours, then the drying process was repeated until the two successive weights were obtained with a difference of less than 0.5 mg (8).

## Ash content

A sample of five grams sample was placed in a heating Carbolite muffle furnace at 525°C for approx. 16 hours, or until it is carbon-free as shown by the white or grey ash residue (9).

## **Protein content**

It was estimated according to Kjelhal method, where 30 ml sulphuric acid were added to one gram sample and digested in a Kjeldahl unit for two hours using copper sulphate as a catalyst. The digest was neutralized with 0.1N Sodium hydroxide to liberate ammonia that was distilled off, collected in boric acid and then titrated with 0.02N hydrochloric acid. The nitrogen percent was calculated and multiplied by 6.38 to get protein content in infant milk formula (9).

### Fat content

Fats were separated from infant formulas using Gerber method where 2.0 g were mixed with 10 ml sulphuric acid, 1.5 ml amyl alcohol and 10 ml distilled water, and then centrifuged for 15 minutes at 65°C. The fat content was read directly from the graduated part of the butyrometer (10).

### **Carbohydrate content**

Carbohydrate contents of infant milk formulas were determined by subtracting moisture, fat, protein and ash percent of each sample from 100 (9).

#### **Minerals content**

Five-grams sample was dry ashed in a furnace at 450°C for about 8 hrs until a white or grey ash residue was obtained. The ash was dissolved in a 10 ml of 10% HCL, and then topped up to 50 ml with distilled water. A blank control (solvent alone) and three standards for each metal (1.00, 1.50, and 2.00 ppm) were diluted with distilled water into 50 ml volumetric flask. After its calibration with the standards, Atomic Absorption Spectrophotometer was used for measuring mineral contents in infant formulas and blank controls (11).

#### Vitamins content

The contents of vitamins A (retinol acetate), vitamin D (cholecalciferol), Vitamin E (DLa-tocopherol) were determined using the HPLC where four ml of n-hexane was added to one g of the sample in a screw-capped extraction tube and flushed with a steam of nitrogen. After centrifuging the mixture at 4000 rpm for 5 min, 1 ml of the supernatant was evaporated under nitrogen to remove the solvent. The residue was re-dissolved in 0.3 ml n-butanol before being injected into the HPLC (12).

## **Statistical analysis:**

Data was statistically analyzed using SPSS program version 16.0. The cutoff point for statistical significance was *P* value  $\leq 0.05$  and all tests were two-sided. Data was tabulated and presented in the form of arithmetic mean and standard deviation for proximate principles and mineral contents. Kruskal-Wallis was used as test of significance (13).

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## RESULTS

Proximate principles contents of Infant milk formulas in table (1) showed that the highest contents were in ordinary formulas except for moisture content where lactose free formula had the highest percentage  $(2.55\pm0.02)$ . The lowest protein and fat contents were in ordinary formula (brand 3) whereas extra care formulas had the lowest ash and lactose free formula had the lowest moisture content. There were significant variations among ordinary infant formulas (brand 1 and 2) in their protein and moisture contents and among lactose free formulas only in their protein contents. Actual moisture contents complied with the contents declared by the manufacturer for all analyzed formulas except for ordinary formula (brand 1). Protein and fat contents were lower than that declared on the label in all formulas except in ordinary formula (brand 2) and lactose free formula in their protein contents. On the contrary, the actual carbohydrate contents were higher than that declared by the manufacturer for all analyzed formulas.

Mineral contents of infant formulas as shown in table (2) revealed that the highest contents were in ordinary formulas specially (brands 1 and 2), except for iron where lactose free formula had the highest contents ( $6.35\pm0.04$  ppm). The lowest mineral contents were in extra care formulas except for sodium and potassium where ordinary formula (brand 1) had the lowest contents. There were significant variations among infant formulas in their calcium, sodium, zinc and copper contents. The actual mineral contents were higher than that declared by the manufacturer in all formulas except in ordinary formulas (brands 2 and 3) and extra care formulas in their iron contents and ordinary formulas (brand 2). The actual copper contents were lower than that declared by the manufacturer except for (brand 1).

Vitamin contents of infant formulas as shown in table (3) revealed that the highest contents of vitamins A, D and E were in ordinary formulas (brand 1), ordinary formulas (brand 2) and extra care formulas (587.0  $\mu$ g, 10.68 mg and 15.2  $\mu$ g/100 g; respectively), whereas the lowest contents were in extra care formulas except for vitamin E where ordinary infant formula (brand 1) had the lowest contents. The actual vitamin contents were lower than that declared by the manufacturer in all formulas except for ordinary formulas (brand 1) in their vitamin A and D, ordinary formulas (brand 2) in their vitamin D and E contents and except for extra care in their vitamin E contents. Moreover, vitamins A and D were not detected in ordinary formula brand 3.

# DISCUSSION

Infant formulas are described as breast-milk substitutes that are specially manufactured to satisfy the nutritional requirements of infants and are based on the composition of human milk, which is the ideal food for infants (14). The higher carbohydrate level in human milk plays a significant role in infant nutrition. Human breast milk contains both digestible and indigestible carbohydrates, while infant formulae, only digestible carbohydrates are required and regulated (15). The present study revealed that carbohydrate contents of infant formulas ranged from 57.14 % to 71.65%. The actual

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carbohydrate contents were higher than that declared by the manufacturer for all analyzed formulas. Another study comparing carbohydrate contents of infant formulas between developed and developing countries reported more or less similar (58.42% in formulas collected from developing countries and 68.0% in formulas from developed countries (16). Carbohydrate contents of infant formulas set in Codex Alimentarius range between 9 and 14.0 g/100 kcal (about 60.0 to 93.3 %) (17).

During infancy, high amount of protein is required because it is essential for normal growth, body development and tissue repair. The present study revealed that protein contents differed significantly among most of the examined formulas and ranged from 6.06% to 11.71%. Moreover, the actual protein contents were lower than that declared on the label in all formulas except in ordinary formula (brand 2) and lactose free formula. Another study reported protein contents of 11.63% in formulas collected from developing countries while they were 12.14% in formulas collected from developed countries (16). Protein contents of infant formulas set in Codex Alimentarius range between 1.8 and 3.0 g/100 kcal (about 12.0 to 20 %) (17).

Dietary lipids are indispensable for normal growth and development. Besides energy, they provide fat-soluble vitamins and are necessary for efficient absorption of fat-soluble vitamins, carotenoids, and cholesterol (18). The present study showed that fat contents of infant formulas ranged from 18.70 to 26.70 %. The actual fat contents were lower than that declared on the label in all formulas. A wider range was reported by another study (3.86 and 29.83%) (16). Fat contents of infant formulas set in Codex Alimentarius range between 4.4 and 6.0 g/100 kcal (about 29.3 to 40.0 %) (17).

The present study revealed that moisture contents of infants milk formulas ranged from 0.42 to 2.55 % with significant differences among samples collected from ordinary formulas (brand 1) and (brand 2) ( $P \le 0.05$ ). Moreover, the actual moisture contents were within the values declared on their labels. Another study comparing moisture contents of infant formulas between developed and developing countries reported higher moisture contents (3.66% and 2.62%; respectively) (16). Moisture contents were below the maximum level recommended by Codex standard for infant formulas (5%) (17).

The present study revealed that ash contents of milk formulas ranged from 2.19 to 3.39 % in extra care formulas and ordinary formulas (brand 1); respectively with non-significant differences in ash contents among samples collected from different types of milk formulas (P > 0.05). The actual ash contents were higher than that declared by the manufacturer for all analyzed formulas. Another study reported more or less similar range (2.04% to 3.09%) (16).

Among the nutrients available (e.g., protein, lipids, and carbohydrates) in infant formulas, minerals are essential for biological processes and play a vital role in normal growth and development (19). Their deficiency or excess may induce adverse effects especially in children (20). Sodium is an electrolyte that carries an electric charge throughout the body, manages blood volume and pressure, and helps to control absorption of other nutrients. Sodium levels in babies can fluctuate, especially soon after birth when a newborn's bodily systems are beginning to function on their own (21). The present study revealed that sodium contents in infant formulas ranged between 620.15 and 1168.15 ppm with significant differences in sodium contents among samples collected from different types

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of milk formulas ( $P \le 0.05$ ). Actual sodium contents were about two to seven times more than those declared on the label. Another study revealed that sodium contents ranging between 1100 and 2100 mg/l (16). Sodium contents of infant formulas set in Codex Alimentarius range between 20.0 and 60.0 mg/100 kcal (about 133.2 to 399.7 ppm) (17). Infant formulas contain higher calcium than that provided through h the mother's milk because the cow milk used in formulas contains higher concentrations (11, 22), despite lower calcium bioavailability in infant formulas (38 % compared to 58 % in mother's milk (16,23). The present study showed that calcium contents ranged from 439.47 to 727.39 ppm with significant differences in calcium contents among samples collected from different types of milk formulas ( $P \le 0.05$ ), moreover, actual calcium contents were more than those declared on the label except for ordinary formulas (Brand 2) that showed more or less similar contents. Another study reported calcium range of (2900 and 6700 mg/l) (16). Another study reported that tested formulas contain almost six times of calcium higher than its recommended intake (24) and showed that despite the lower bioavailability of calcium from formulas, there is a risk of exceeding the daily intake which may lead to kidney problems and impair the absorption of magnesium, iron and zinc (25). Codex Alimentarius sets minimum calcium content in infant formulas of 50.0 mg/100 kcal (about 333.1 ppm) (17).

The present study showed that potassium contents ranged from 643.05 to 842.35 ppm with non-significant differences in its contents among samples collected from different types of milk formulas (P > 0.05); moreover, actual potassium contents were more than those declared on the label. A Nigerian study compared potassium contents of milk formulas collected from developed and developing countries showed that potassium contents ranged between 2200 and 5300 mg/l (16), this range is about 4-5 times much higher than that reported by the present study. Another study of different milk formula brands in SPAIN reported a range of potassium contents of (483 -1192 mg/l). Potassium contents of infant formulas, set in Codex Alimentarius, range between 60.0 and 180.0 mg/100 kcal (about 399.7 to 1199.1 ppm) (17).

Magnesium is essential for the proper functioning of the body and its deficiency may lead to sudden infant death. Breast milk, with a Mg content of 34 mg/l, is the best source of this element for infants (24). The present study showed that magnesium contents ranged from 44.62 to 63.67 ppm with non-significant differences in its contents among samples collected from different types of milk formulas (P > 0.05), moreover, actual magnesium contents were more than those declared on the label. The actual magnesium contents were higher than the minimum magnesium content set in the Codex Alimentarius; 5.0 mg/100 kcal (about 33.3 ppm) (17). Another study of Spanish infant formulas reported slightly higher mean magnesium concentration (71.3 mg/l) (26).

With optimal reserves of iron in the newborn, its absorption from breast milk is sufficient to cover the daily requirements. At about 4–5 months of age, demand of tissues for iron increases so formulas should be supplemented with iron. Significant iron deficiency in infants may lead to impaired cell-mediated immunity, while an excess may lead to serious liver damage (26). The present study showed that iron contents ranged from 3.04 ppm in extra care formulas to 6.35 ppm lactose free formulas with non-significant differences in its contents among samples collected from different types of milk formulas

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(P > 0.05), moreover, actual iron contents were lower than those declared on the label except in Ordinary formulas (brand 1) and lactose free formulas. The highest iron content in lactose free formulas may be attributed to their fortification with iron. Other studies reported wider iron ranges (4.3 and 26.6 µg/g) (27), and (1.02–67.5 µg/g) (28). Codex Alimentarius sets minimum iron content in infant formulas of 0.45 mg/100 kcal (about 2.9 mg/l) (17). Zinc is required for the synthesis of protein and nucleic acid metabolism. For infants, the recommended daily Zn intake is 5.0 mg/day. The concentration of Zn in mother's milk is about 2 mg/kg and decreases over time with lactation to reach 20 % of its initial concentration after 3 months (24). The present study showed that zinc contents ranged from 3.59 to 10.59 ppm in extra care formulas and ordinary formulas (brand 1); respectively with significant variations in its contents among samples collected from different types of milk formulas ( $P \le 0.05$ ), moreover, actual zinc contents were higher than those declared on the label where ordinary formulas (brand 1) showed the maximum difference that contains about as three times as declared on the label.

An excess of zinc can lead to microcytic anemia or neutropenia and reduce the concentration of iron in the body. Additionally, zinc competes with magnesium at the absorption level in the intestines and in the structural parts of the bone (24). Another study reported that Zn contents of various brands of infant formulas ranged between 1.72 and 7.81  $\mu$ g/g (27). Codex Alimentarius sets minimum zinc content in infant formulas of 5.0 mg/100 kcal (about 3.33 ppm) (17). The majority of the copper in infant formula is added through the trace element premix during the manufacturing process, in addition to copper from milk or whey protein (27). The present study revealed that copper contents ranged from 0.163 ppm in extra care formulas to 0.367 ppm in ordinary formulas (brand 1) with significant variations in its contents among samples collected from different types of milk formulas ( $P \le 0.05$ ), moreover, actual copper contents were lower than those declared on the label except in ordinary formulas (brand 1). Another study reported a lower copper range amongst the analysed infant foods (from not detected to 0.100 mg/g) (27-29). Codex Alimentarius sets a minimum copper content in infant formula of 35.0  $\mu$ g/100 kcal (about 0.233 ppm) (17).

Vitamin A is essential for the maintenance of healthy vision, healthy skeletal and tooth development, cellular differentiation and proliferation, reproduction and integrity of the immune system (30). The present study revealed that Vitamin A contents ranged from 30  $\mu$ g/100g in extra care formulas to 587 mg/100g in ordinary formulas (brand 1), moreover, the vitamin was not detected in ordinary formulas (brand 3). The actual Vitamin A contents were lower than those declared on the label except in ordinary formula (brand 1). Another study reported values of vitamin A (as retinyl acetate) in the range 590–740  $\mu$ g /100 g in four types of Infant Formulas that was higher, between 113% and 120%, than those stated on their respective labels (31).

Breast-fed infants are at risk for vitamin D deficiency due to limited amounts of vitamin D in breast milk and limited exposure to sunlight (32). The present study revealed that Vitamin D contents ranged from 2.66 mg/100g in extra care formulas to 10.68 mg/100g in ordinary formulas (brand 2); moreover, it was not detected in ordinary formulas (brand 3). The actual Vitamin D contents were higher than those declared on the label in ordinary formulas. Another study reported that most of the analyzed infant formulas had

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higher fat-soluble vitamin contents than those declared and attributed it to the desire of the manufacturers to ensure that at the end of the shelf-life of the formula, vitamin contents are at least as high as the label states (33).

Vitamin E has antioxidant activity, immunomodulation and is the most potent lipid peroxyl radical scavenger that protects tissues against chromosome damage and DNA oxidation (34). Tocopherols are added to infant formulas because they increase vitamin content and prevent lipid peroxidation therefore, prolonging the product's shelf life (35). The present study revealed that Vitamin E contents ranged from 0.71  $\mu$ g/100g in ordinary formulas (brand 1) to 15.2  $\mu$ g/100g in extra care formulas. The actual Vitamin E contents were lower than those declared on the label except in ordinary formulas (brand 2) and extra care formulas. Another study reported that vitamin E ( $\alpha$ -tocopherol) values in the infant formulas were 2-3.5 higher than that declared on the label (35).

## CONCLUSION

There were discrepancies between the actual chemical composition of infant formulas and those declared by the manufactures on their labels. Further studies are required to evaluate the chemical composition of infant formulas on a greater number of samples and a wider diversity of brands to ensure the accuracy of the contents declared on their label.

## **Conflicts of Interest**

The authors declare no conflict of interest.

## **Author Contributions**

All authors have made full contribution to data acquisition, interpretation of results, drafting and revising the final manuscript. All authors read and approved the final manuscript.

## **Study Limitations**

Limited number of infant formula samples were collected and analyzed due to financial constraints.

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# Table 1: Proximate principles contents (%) of Infant milk formulas ascompared to those declared on their label

	Degualda	Infant Milk Formulas							
Element	Kesuits		Ordinary #	Lactose	Extra				
	source	1	2	3	free	care			
Carbohydrates	Analysis	58.28	58.43	71.56	57.14	64.68			
	Label	54	56.9	53.6	57.1	53.4			
Protein	Analysis	11.42±0.42*	11.71±0.39*	$6.06 \pm 0.09$	11.12±0.20*	$7.30 \pm 0.24$			
	Label	12.5	11.5	10.3	10.3	9.6			
Foto	Analysis	26.7	25.3	18.7	26.6	23.3			
rais	Label	28	26	24	27.3	25.5			
	Analysis					2.53			
Moisture		$0.42\pm0.10^*$	$1.18 \pm 0.10*$	$1.49 \pm 0.08$	$2.55 \pm 0.02$	±0.04			
	Label	2.5	< 3	<b>≤</b> 3	3	$\leq 3$			
Ash	Analysis	$3.18 \pm 0.05$	$3.39 \pm 0.03$	$2.20\pm0.10$	2.52±0.10	$2.19 \pm 0.05$			
	Label	3	2.5	ND	ND	ND			

<sup>#</sup>From three commercial brands (1, 2 and 3)

 $^{*}P \leq 0.05$ 

ND: Not declared on the label

Table 2: Mine	ral	contents	(ppm)	of	Infant	milk	formulas	as	compared	to	those
declared on the	ir l	abel									

	Degulta	Infant Milk Formulas									
Mineral	source		Ordinary #	T 4	<b>D</b> =-4						
		1	2	3	Lactose free	Extra care					
	Analysis	727.39									
Ca		$\pm 193.57$	$509.00 \pm 52.09$	$452.64 \pm 30.60$	$505.36 \pm 23.25$	$439.47 \pm 9.57$					
	Label	500	510	368	428	343					
P value*		$P \le 0.05$									
V	Analysis	643.05±21.65	842.35±10.55	655.10±30.60	713.90±60.55	741.23±63.66					
N	Label	550	500	505	506	494					
P value*		P > 0.05									
Na	Analysis	620.15±54.65	1168.15±223.95	862.15±113.75	1024.55±153.75	1098.50±89.8					
	Label	180	183	144	132	138					
P value*		$P \le 0.05$									
Ma	Analysis	60.80±17.97	63.67±5.19	55.01±2.59	56.73±0.92	44.62±2.12					
wig	Label	36	47	37	40	37					
P value*		P > 0.05									
Fe	Analysis	$5.70 \pm 0.08$	5.15±0.03	$5.00 \pm 0.02$	6.35±0.04	3.04±0.03					
	Label	5	6.1	5.3	6.2	3.9					
P value*		<i>P</i> > 0.05									
Zn	Analysis	$10.59 \pm 0.31$	7.168±0.07	$4.005 \pm 0.02$	8.404±0.16	3.591±0.03					
	Label	3.8	4.8	3.3	4.4	3.4					
P value*		$P \le 0.05$									
Cu	Analysis	0.367±0.004	0.324±0.004	0.203±0.001	$0.289 \pm 0.002$	0.163±0.001					
	Label	0.35	0.329	0.298	0.327	0.293					
P va	lue*	$P \leq 0.05$									

<sup>#</sup> From three commercial brands (1, 2 and 3) <sup>\*</sup> *P* value among various analyzed contents of infants milk formulas

# Table 3: Vitamin contents ( $\mu g/100g$ ) of Infant milk formulas as compared to those declared on their label

	Degualda	Infant Milk Formulas							
Vitamins	Kesuits	(	Ordinary #		Lastaga fuas	Extra care			
	source	1	2	3	Lactose free				
Vitamin A	Analysis	587.0	405.0	ND	374.0	30.0			
	Label	540	479	367	428	363			
Vitamin D	Analysis	9.18	10.68	ND	5.16	2.66			
(mg/100g)	Label	9	10	9	9.2	8.7			
Vitamin E	Analysis	0.71	10.8	5.63	4.48	15.2			
	Label	1.5	4.8	7.7	8.6	7.3			

<sup>#</sup> From three commercial brands (1, 2 and 3)

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