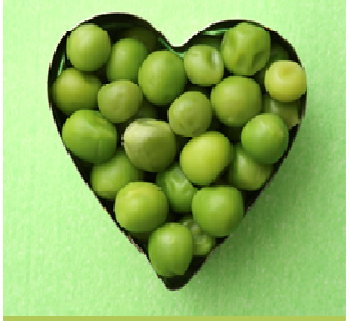
NUTRITIONAL STATEMENT

Nutritional value of Pisane®



December 2013

DEFINITIONS

Different parameters are used to investigate the nutritional value of a particular protein. They are listed below.

- Amino acid score (AAS) :percentage of most limiting essential amino acid concentration in the food protein in comparison with the same amino acid concentration in the reference pattern

- True digestibility: The proportion of food nitrogen that is absorbed, as determined by the rat balance method. It is determined by the following formula:

$$True\ digestibility = I – (F\text{-}Fe) / I$$

- Biological value (BV): proportion of absorbed nitrogen that is retained in the body. It is determined by the following formula:

$$BV = I – (F\text{-}Fe) - (U\text{-}Ue) / I – (F\text{-}Fe)$$

- Net protein utilization (NPU): nitrogen retention or proportion of nitrogen intake that is retained in the body. It is determined by the following formula:

$$NPU = I – (F\text{-}Fe) - (U\text{-}Ue) / I$$

- Protein Efficiency Ratio (PER) : weight gain of a growing rat divided by its intake in protein. This value is sometimes used as an indication of the protein quality.

Abbreviations

I = intake nitrogen

F = fecal nitrogen

Fe = endogenous fecal nitrogen

U = urinary nitrogen

Ue = endogenous urinary nitrogen

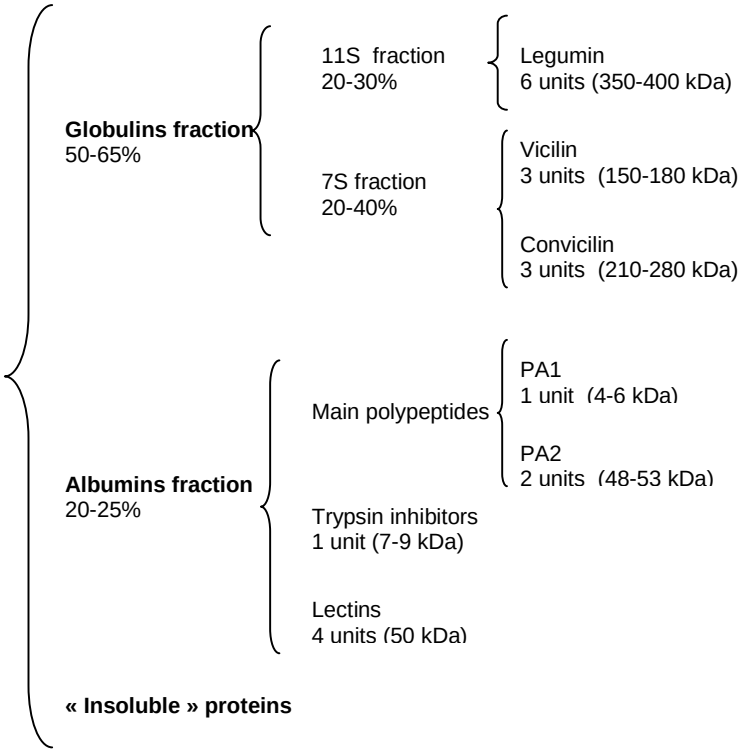
PROTEIN FRACTIONS

Pisane® is a pea protein isolate of high purity extracted from yellow pea (*Pisum sativum*).

Pea is a plant from the leguminous family. All peas that are consumed today (green peas, split peas, dry peas) belong to the *Pisum sativum* species, whatever their color is (which can vary from yellow to green) and whatever their appearance at maturity is (smooth or wrinkled). Leguminous plants have the unique natural capacity to fix nitrogen (used for protein synthesis) from the air thanks to a symbiotic relationship with microorganisms present on their plant roots. They require few chemical fertilizers, providing a direct environmental benefit. Furthermore, pea cropping allows the reduction of the chemical fertilization for the following crops during culture rotation.Besides, water requirements for pea growth are low and its cropping requires a limited amount of crop protection products. Finally, pea is one of the most effective and sustainable ways to produce proteins in terms of water requirements and ground surface consumption.

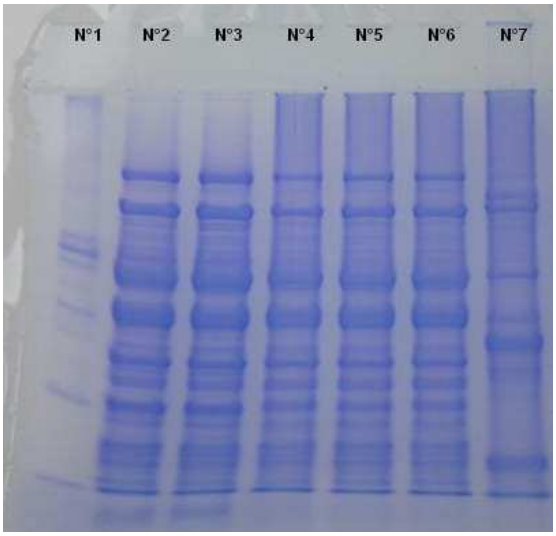
Pea protein in raw pea can be separated in 3 main categories : Globulins, Albumins and “Insoluble” proteins. There is a certain variability in the levels of the various fractions. The globulins fraction can be subdivided in 2 parts (according to molecular mass) : the 11S and the 7S fractions. The albumins fraction can be subdivided in 3 parts (Crevieu-Gabriel 1999).

Figure 1-Protein fractions in raw pea from Crevieu-Gabriel, 1999



Protein fractions found in pea protein isolate are similar to those found in raw pea as illustrated by the electrophoresis analysis presented below in figure 2 (samples N° 2 and 3 : raw pea; samples N°4, 5 and 6 : pea protein isolate Pisane®). On contrary, soy protein isolate (sample N°7) exhibits a very different profile.

Figure 2-Electrophoresis of proteins contained in raw pea (samples N°2 and 3), pea protein isolate Pisane® (samples N°4, 5 and 6) and soy protein isolate (sample N°7)



AMINO ACID SCORE

Pisane® has a well-balanced amino-acid score. Among the nine essential amino-acids, only one is slightly below the FAO 2007 recommendations from the FAO (see table below).

Table 1- Essential Amino Acids composition of Pisane® compared to the FAO suggested amino acid pattern for adults over 18 years (2007)

Essential Amino Acids	FAO suggested amino acid pattern for adults > 18 y (2007)	Pisane® (g/100g protein)
Histidine	1.5	2.5
Isoleucine	3	4.5
Leucine	5.9	8.4
Lysine	4.5	7.2
Methionine+Cystine	2.2	2.1
Phenylalanine+Tyrosine	3.8	9.3
Threonine	2.3	3.9
Tryptophane	0.6	1.0
Valine	3.9	5.0

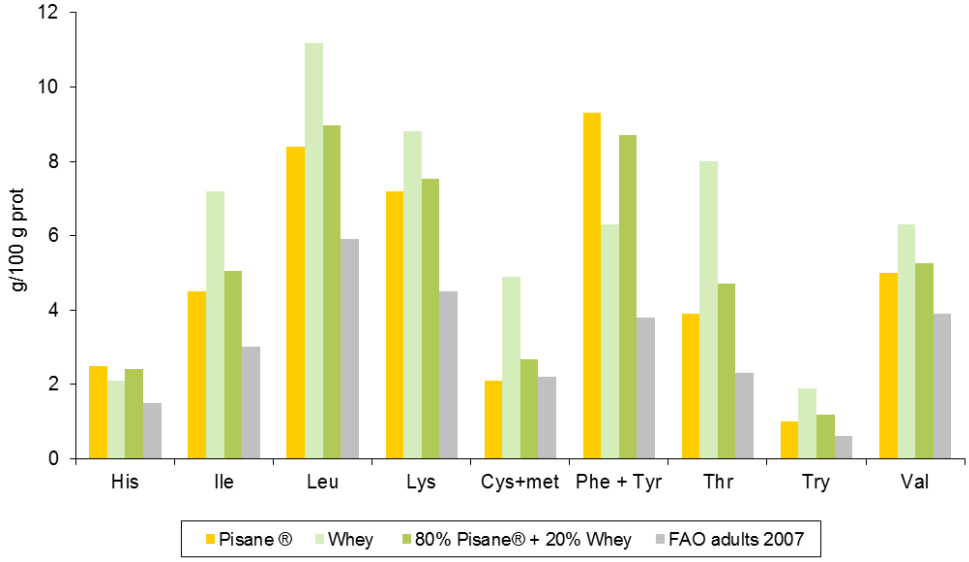
The **Amino Acid Score (AAS**) of Pisane® is **0.96** based on the FAO suggested amino acid pattern for adults over 18 years old (2007).

Pisane®, pea protein isolate, can be combined with other sources of proteins in order to reach an optimal AAS of 1 as illustrated in the examples below.

Combination with animal protein sources

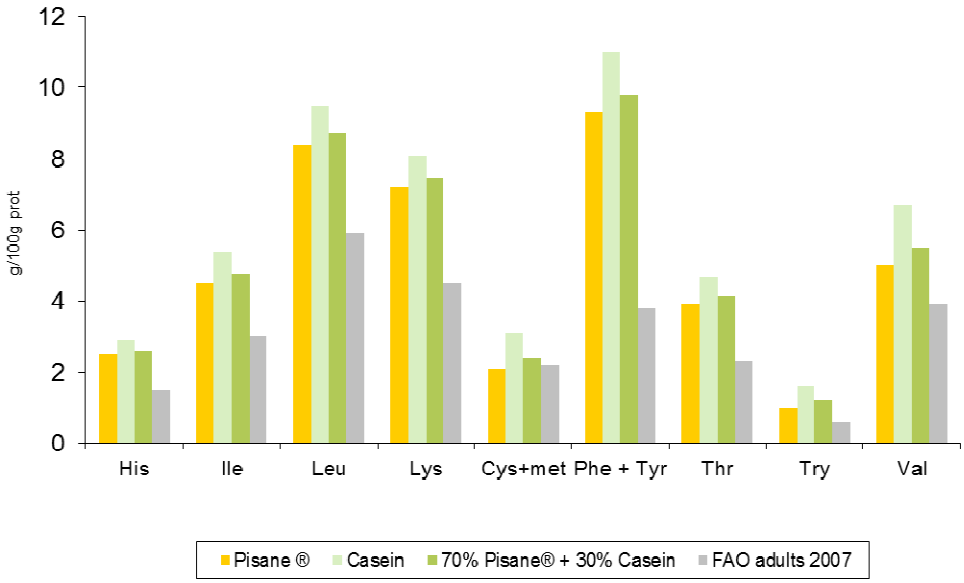
Whey protein isolate

Figure 3- Essential Amino Acid profile of a mix composed of 80% Pisane® and 20% whey protein isolate



Casein

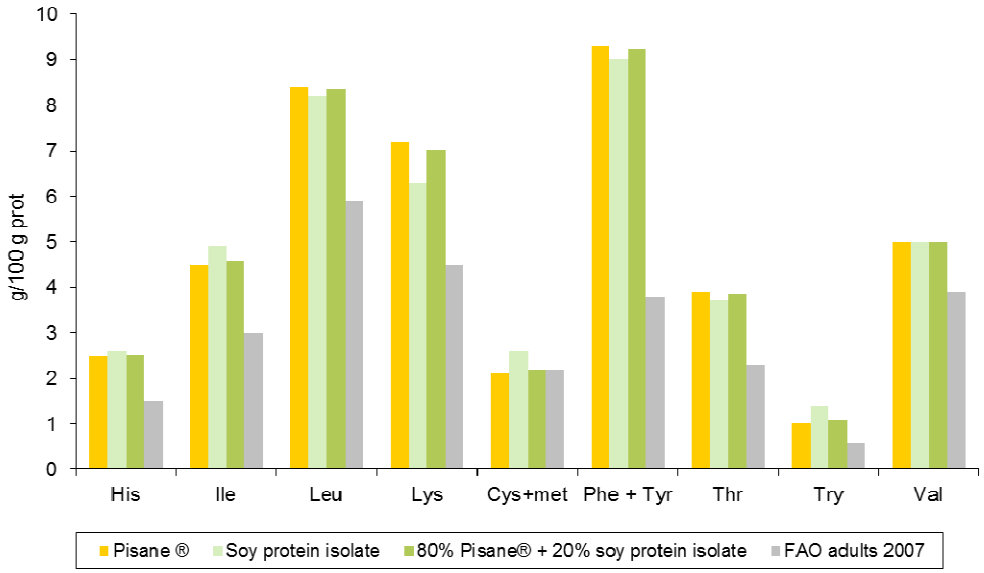
Figure 4- Essential Amino Acid profile of a mix composed of 70% Pisane® and 30% casein



Combination with other vegetable protein sources

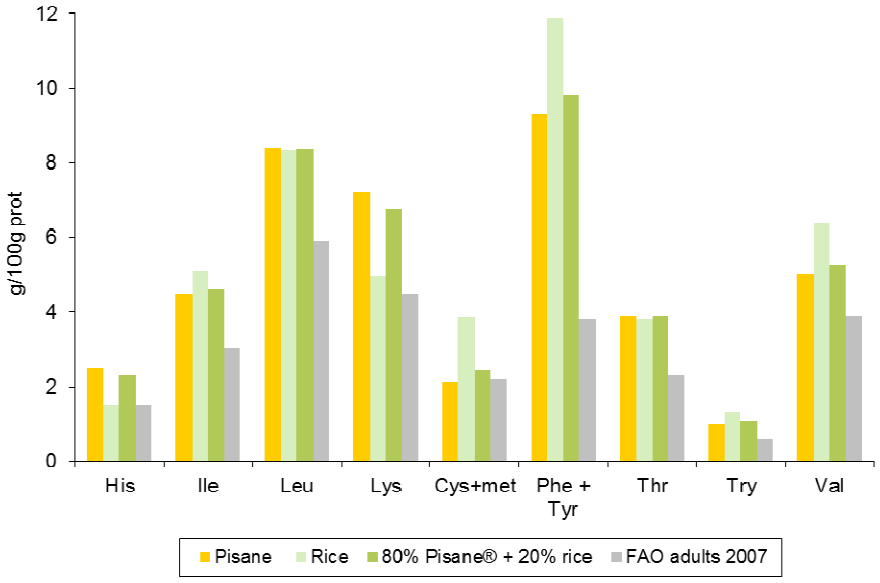
Soy protein isolate

Figure 5- Essential Amino Acid profile of a mix composed of 80% Pisane® and 20% soy protein isolate



Rice protein

Figure 6- Essential Amino Acid profile of a mix composed of 80% Pisane® and 20% rice



TRUE DIGESTIBILITY

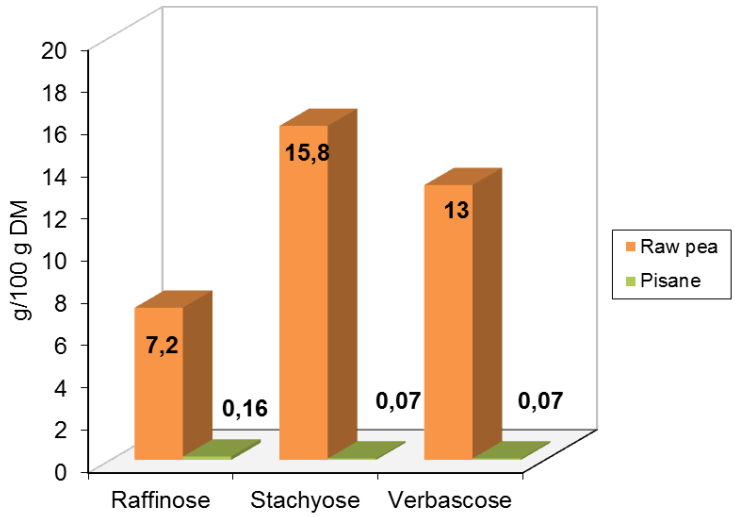
The true digestibility of Pisane® has been measured in a rat study conducted by the Pr Tomé (INRA, France) in 2002 (Confidential report Cosucra). The **true digestibility of Pisane® is very high (98%)** and identical to the one of total milk proteins. Comparable value are also found for beef and soy proteins.

ANTI-NUTRITIONAL FACTORS

Those compounds from various origin, naturally presents in leguminous, like pea, soy etc…, can interfere negatively with the proteins digestion. Fortunately, they are **strongly desactivated** by Pisane® production process.

Raw pea contains, for example, significant levels of **alpha-galactosides** (Vidal-Valverde, Frias et al. 2003), also known as "flatulent sugars" which are only found in limited amount in the pea protein isolate Pisane®.

Figure 7- Alpha-galactosides in raw pea and in Pisane®



Trypsin inhibitor, which inhibits proteases by binding them in the intestinal tract, leading to endogen losses of protein and reduction of their digestibility, is also strongly decreased by Pisane® production process . Trypsin inhibitor activity (TIA) of Pisane® has an average value of 3.1 ± 0.8 TIU/mg DM. It has been reported that the value of TIA varies for different pea lines and it can go up to 8.4 ± 0.2 TIU/mg DM in some peas.

Phytic acid (which binds with cationic groups of protein reducing their availability) is present in very limited quantities in Pisane®. **Lectins** (carbohydrates binding proteins of non-immune origin) are even totally absent. Moreover, pea varieties (white flowers) grown in France, such as the ones used for Pisane® production, do not contain **tannins** which are also considered as anti-nutritional factors.

Table 3- Phytic acid and lectins levels in Pisane®

Phytic acid (% on DM)	1-2%
Lectins (Hu/mg DM)	0

ISOFLAVONES CONTENT

Isoflavones are naturally occuring plant chemicals belonging to the phytoestrogen class. Those components are found in relatively high concentration in soybeans and soy-protein products. Data from the literature report that raw peas contain levels of isoflavone 1000 times below the ones found in soy (Mazur, Duke et al. 1998).

Analysis conducted on Pisane® show that its isoflavone content is below the analytical treshold of 3 ppm and far below the content usually found in soy protein isolate.

Table 4- Isoflavones levels found in soy protein isolate and in Pisane® (in mg/kg)

Pisane®	< 3
Soy isolate	± 100

BV, NPU & PER

Fernandez-Quintela et al. have studied the digestibility of pea protein isolate from *Pisum sativum L.* in rats and have found a **Biological Value (BV)** of 53.2% and a **Net Protein Utilization** (**NPU)** of 49.2% (Fernandez-Quintela, Del Barrio et al. 1998).

In a clinical study, Gausseres et al. found a BV of 78% for pea protein in healthy humans (Gausseres, Mahe et al. 1997). This high BV was confirmed by Mariotti et al. who reported a BV of 78.7% and a NPU of 70.9% for a pea protein isolate in healthy adult volunteers (Mariotti, Pueyo et al. 2001). This means that the **nutritional quality of pea protein isolate is very similar to that of soy protein isolate** measured in humans for which a BV of 80.1% and a NPU of 73.3% were reported (Mariotti, Mahe et al. 1999). For comparison, the BV of casein is 80% and that of whey protein is 100%.

Protein Efficiency Ratio (PER) has also been evaluated in two animal studis. In one study, PER value was found to be 1.83 +/- 0.11g weight gain/g protein consumed (Tomé, 2007). In the other one it was found to be 1.5 g weight gain/g protein consumed (Fernandez-Quintela, Del Barrio et al. 1998).

Table 5- PER, NPU and BV of pea protein, data from animal and human studies

Reference	Type	PER	NPU	BV
(Fernandez-Quintela, Del Barrio et al. 1998)	Animal (rats)	1.5	49.2%	53.2%
(Tomé 2007)	Animal (rats)	1.83	50.4%	53%
(Gausseres, Mahe et al. 1997)	Human	-	-	78%
(Mariotti, Pueyo et al. 2001)	Human	-	70.7%	78.7%

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