



IMPACTING
BETTER FOOD



ROVITARIS[®]

Binding Solution

Powered by

 **Rubi Protein[™]**

**A revolutionary clean label binding solution
for plant-based meat and seafood applications**





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The Clean Label Binding Challenge

In recent years, consumer demand for healthier and more sustainable food options has driven the rapid growth of plant-based products in both the retail and food service sectors. Since the 1970s, when the first plant-based companies were established, the market for alternative proteins has gained momentum and encountered challenges as health conscious consumers aren't willing to compromise on factors such as nutrition, flavor, and texture.

To emulate the taste and texture of traditional meat and seafood products, plant-based foods frequently rely on chemically modified additives such as the binding agent methylcellulose. These additives bind the different components of a plant-based product, such as texturized vegetable proteins (TVP) with oil, water, colorants and flavors. These binding agents also enhance emulsion stability, improving textural attributes, which is crucial for shaping and forming the final products. Additionally, they can prevent undesired oil and water leakage during cold storage and transportation, ensuring product integrity.

Beyond Methylcellulose

Methylcellulose is commonly used in plant-based meat formulations to thicken water-based liquids at cold temperatures and form a reversible gel upon heating. This property makes it indispensable for product binding, imparting bite firmness during hot consumption. However, upon cooling, the firmness decreases, which presents a limitation when replicating the textures of traditional meat products.

Not only does it present limited functional properties, it is also chemically derived from cellulose through the process of etherification. Methylcellulose's reputation as a highly processed ingredient (European food additive number is E461), combined with its medical usage as a laxative, has created a negative perception for its use in food applications. Health-conscious consumers seeking label-friendly ingredients are now avoiding products made with methylcellulose, pushing the food industry to develop alternative binding solutions.

While some replacements have become available, these solutions also bring complex formulation challenges. Incorporating these alternatives often calls for significant adjustments to recipe formulations, including the addition of more chemical additives or hydrocolloids. These changes can result in longer ingredient lists that clash with consumers' desire for clean label products. Furthermore, marketed alternatives to methylcellulose tend to come with functional limitations that compromise product texture, often leading to a gummier or mushier consistency.

In this whitepaper, we introduce ROVITARIS® Binding Solution powered by Rubi Protein™, a one-of-a-kind clean label binder developed in partnership with Plantible. We explore the excellent nutritional and functional properties of this clean label replacement that make it a better option to methylcellulose in plant-based meat and seafood applications.



Lemna Leaf Protein

Lemna, also known as duckweed or water lentil, is a floating aquatic plant that has emerged as a highly sustainable source of functional food protein.

Lemna showcases an extraordinary capacity for proliferation in nature, with its biomass doubling within 16 to 48 hours.¹ In addition, its ability to achieve such rapid growth is minimally reliant on environmental conditions, demonstrating its resilience and adaptability. Lemna grows on non-arable land and uses 5-10 times less water than other established protein sources. With changing climate conditions, the yield of current established crops such as soy, maize, and wheat have seen a reduction. Due to this, there is a growing need for a new, resilient protein source to protect the food supply. One of the main advantages of lemna lies in its protein productivity, surpassing that of traditional soybean plants by a significant margin; the elevated total protein output per unit of cultivation area of lemna reaches levels 2-10 times greater than that of soybean plants.²⁻⁵ The total protein content of this plant is 30-45% of its dry weight.^{6,7} This elevated protein content renders lemna a compelling sustainable nutritional resource and the protein isolated from lemna leaf is among the most functional within commercially available proteins. This makes lemna particularly important in the agriculture and food industry.

RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is a prevalent protein in lemna leaf and is known as the most abundant protein on Earth due to its high presence in all green foliage.⁸ In addition to this ubiquity, RuBisCO's emergence as a sought-after food ingredient stems from its remarkable nutritional value, low allergenicity and unique functional attributes. Efforts to incorporate this protein into the food supply, however, have been hindered by the challenge of isolating and purifying it from green leaf plants while retaining its functionality as well as eliminating undesirable flavors and green chlorophyll. This challenge persisted until recent advances in technology by Plantible Foods with its cutting-edge Rubi Protein™ RuBisCo.

Rubi Protein™, a critical component of the ROVITARIS® Binding Solution, has a label-friendly declaration of lemna leaf protein. With gelling, foaming, emulsifying, and other functional properties, Rubi Protein™ enables the replacement of food additives, egg white and other animal proteins to improve the taste, nutritional profile, and sustainable production of bakery, beverage, dairy, and plant-based meat and seafood applications.



How Rubi Protein™ Works in the ROVITARIS® Binding Solution

Nutritional Quality

The use of available plant proteins in food formulations is often hindered by inherent nutritional limitations.⁹ Cereals, seeds, and nuts are often lacking in lysine, while legumes contain lower levels of cysteine and methionine. In contrast, the amino acid profile and Digestible Indispensable Amino Acids Score (DIAAS) of Rubi Protein™ (109%) within ROVITARIS® Binding Solution aligns with the protein quality outlined by the FAO, rivalling and surpassing animal proteins like casein (105%, Fig. 1).¹⁰⁻¹² The digestibility kinetics of Rubi Protein™ were evaluated using tiny-TIMsg - a smart artificial gut designed to mimic the upper gastrointestinal tract. The results confirmed that Rubi Protein™ was as easily digested as casein.

Comparison of the DIAAS Scores

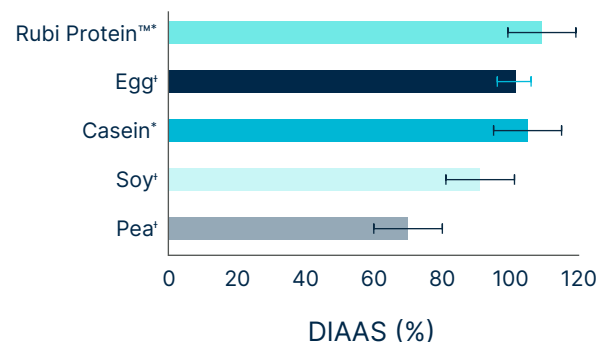


Figure 1: DIAAS is calculated by dividing the DIAA of sample with DIAA of FAO reference protein and multiplying by 100 to express as a percentage. *Data from tiny-TIMsg evaluation by TIM BV for ICL. †Data from references.^{13,14}



Functional Properties

Rubi Protein™ in ROVITARIS® Binding Solution is unique in its ability to be solubilized and to gel. The protein is highly soluble across a wide pH range with a percent solubility of >95% at neutral pH in water. It can form an irreversible gel in water at temperatures as low as 60°C and a concentration as low as 2% w/w. Rubi Protein™ exhibits the highest gel hardness value among food proteins, in the presence (221 g) or absence of salt (125 g), at a 4% protein concentration (w/w, Fig. 2). It surpasses the egg white gel hardness (45 g) at the same concentration. In contrast, other plant proteins from soy, pea, and mung bean are unable to form self-supporting gels at 4% w/w. While leveraging the excellent solubility and gelling benefits of Rubi Protein™, the ROVITARIS® Binding Solution is a customized solution, formulated to provide additional benefits to meet the specific needs in plant-based meat and seafood applications.

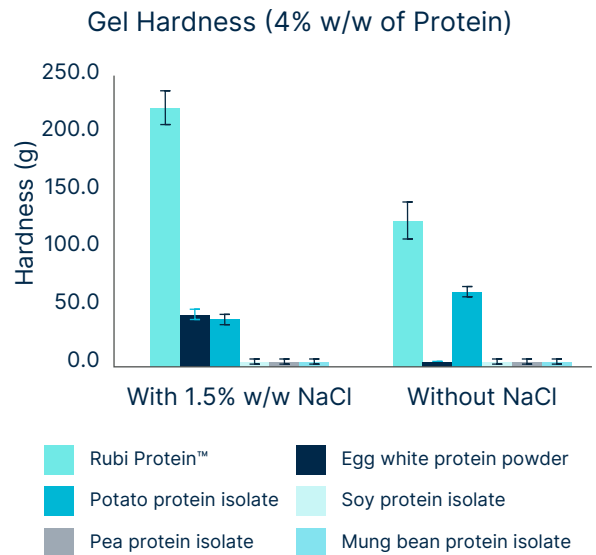


Figure 2: Hardness value is used to determine the gel strength of proteins. The measured force is commonly correlated with the force required to compress the food between molars during mastication. Gel hardness was calculated from the end point of the first compression assay using TA.XTplus, Stable Micro Systems.

ROVITARIS® Binding Solution in Applications

ROVITARIS® Binding Solution powered by Rubi Protein™ (RBS) is an easy-to-apply replacement for methylcellulose (MC) in applications involving plant-based meat and seafood. Ingredient composition of the analyzed samples is presented in Table 1, for reference. The percentage of methylcellulose was selected based on common use range for plant-based meat products and in a side-by-side texture comparison with 80/20 beef samples. The suggested concentration range of the ROVITARIS® Binding Solution is 3-6% depending on the desired hardness and application method. Due to the quick hydration and robust binding effect of RBS,

food scientists and product developers have the versatility of simplifying their production steps during ingredient addition for enhanced operational efficiency. Typically, the ingredient addition with MC involves a multi-step process, starting with the activation of MC through high shear mixing in iced water and oil. It is subsequently mixed with the remaining ingredients, then shaped and cooked. Conversely, the ROVITARIS® Binding Solution samples were produced through a simple process of mixing all components in cold water, followed by shaping and cooking. RBS can also be used to create a separate emulsion, similar to MC.

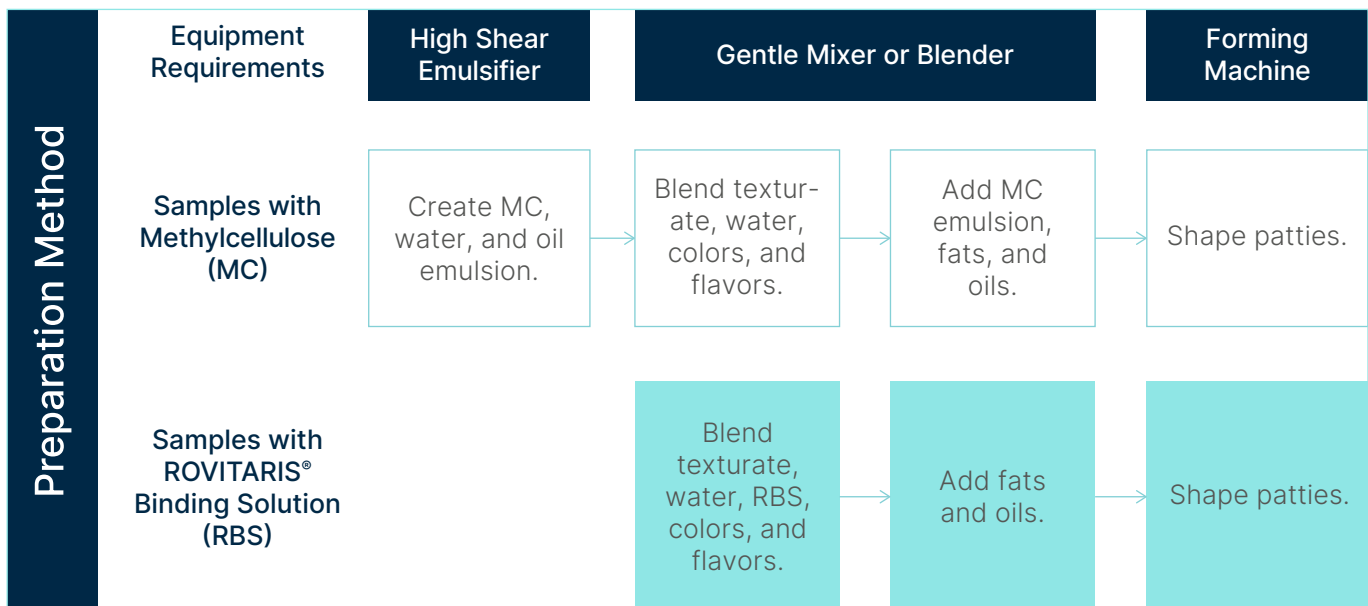
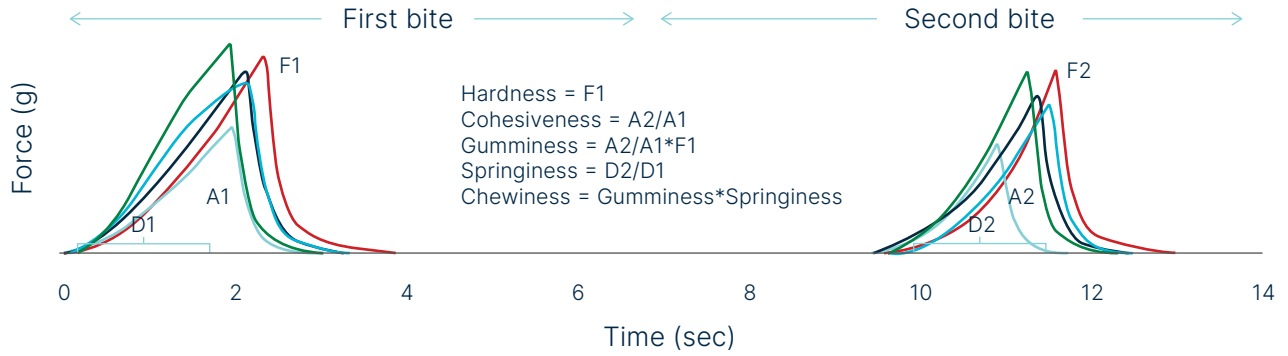


Table 1: Ingredients and their percentages used in application case study.

Ingredient	1% MC	2% MC	5% RBS	6% RBS
Water	59.8	58.8	55.8	54.8
ROVITARIS® FBX360 Textured Fava Bean Protein	28.0	28.0	28.0	28.0
Methylcellulose	1.0	2.0	0.0	0.0
ROVITARIS® Binding Solution	0.0	0.0	5.0	6.0
Salt	1.0	1.0	1.0	1.0
Coconut Fat	3.0	3.0	3.0	3.0
Canola Oil	5.0	5.0	5.0	5.0
Natural Colors	2.2	2.2	2.2	2.2



A



B

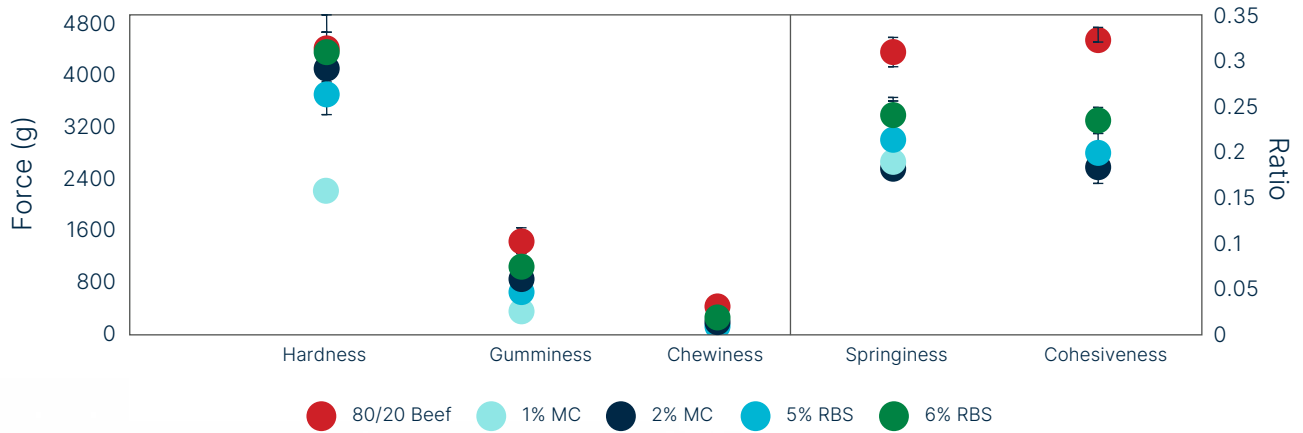


Figure 3: The texture of soft solid foods can be measured instrumentally using Texture Profile Analysis (TPA), which involves a two-cycle compression of uniformly cut samples of the food under investigation. A number of attributes can be calculated from the force-time and stress-strain curves, each of which approximate different aspects of the sensory experience of eating. A) Single measurement examples of TPA curves and calculation of texture parameters. B) Average hardness, gumminess, chewiness values on left axis. Average springiness and cohesiveness on the right axis. Sample measurements were conducted in quadruplicates at warm consumption temperatures.

After forming and chilling, products were cooked in order to evaluate texture quality at consumption temperatures. Texture profile analysis (TPA) was conducted, encompassing attributes like hardness, chewiness, gumminess, cohesiveness, and springiness (Fig. 3B). The hardness value of the products containing 5% or 6% ROVITARIS® Binding Solution was statistically similar to samples containing 80/20 beef (and/or) 2% MC. Gumminess and chewiness describe the energy or work needed to break down food. The corresponding values obtained for RBS samples were closer to those of 80/20 beef compared to samples with 2% MC.

The cohesiveness and springiness values, reflecting the ability of the product to maintain its shape and recover its shape after compression respectively, were highest in 80/20 beef samples, followed by samples with ROVITARIS® Binding Solution. In contrast, MC samples had significantly lower springiness and cohesiveness values, indicating their inability to retain structural integrity and regain shape after compression. These measurements provide valuable insights into the correlation between the product's texture and its oral processing during consumption, demonstrating the successful replacement of methylcellulose with ROVITARIS® Binding Solution by providing comparable ingredient binding and textural quality while improving on key desirable texture attributes, such as springiness and cohesiveness.



A challenge that is often encountered with MC in plant-based meat products is its tendency to have decreased binding and a softer texture at colder temperatures. This trait leads to difficulties in using such formulations in retorted products, which are typically kept at room temperature after heating, or in plant-based cold cuts where product handling occurs in chilled temperatures. In contrast, ROVITARIS® Binding Solution shows the ability to maintain consistent texture and binding across a wide range of temperatures, providing comparable handling to meat products (Fig. 4A). This advancement allows for the use of a single plant-based formulation for both hot and cold applications, including sandwiches, pizzas, salad toppings, soups and more.

Moreover, the capacity of ROVITARIS® Binding Solution to uphold its gelling and binding properties offers an additional advantage by reducing cook loss (Fig. 4B). Unlike products relying on MC as a binding agent, the samples with RBS displayed no cook loss during the cooking period under assessment, leading to an increased yield for ready-to-eat products and improvements in sensory quality, such as flavor and mouth-coating.

Alongside its functional advantages, the ROVITARIS® Binding Solution boasts a high protein content, allowing for the incorporation of additional grams of protein per 100 g when used at the recommended levels for plant-based meat (Fig. 5).

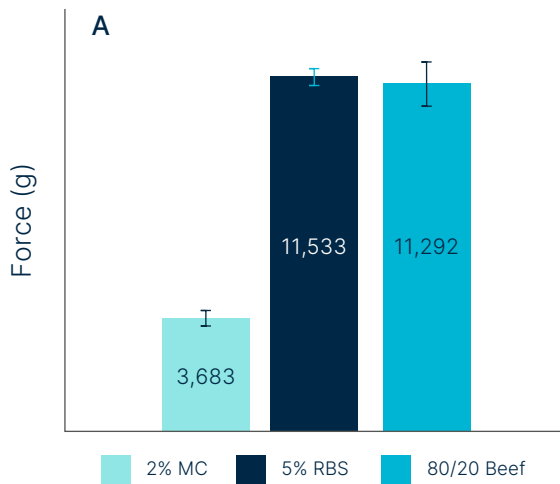


Figure 4A: Cold hardness value was assessed by taking the peak force during the TPA measurement. The samples were kept in a refrigerator after cooking, prior to measurement.

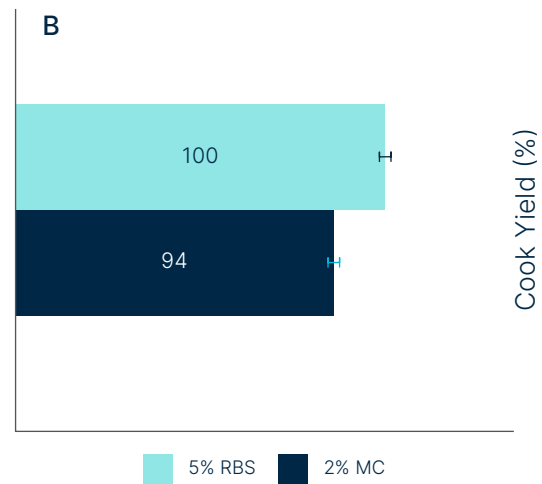


Figure 4B: Cook yield value was obtained by taking the difference in weight of the samples before and after cooking. Samples were cooked in a combination steam oven set at 120°C and 100% steam until a target internal temperature of 71°C was reached.

Methylcellulose

Nutrition Facts	
servings per container	
Serving size	(100g)
Amount per serving	
Calories	190
<small>% Daily Value*</small>	
Total Fat 9g	12%
Saturated Fat 3g	15%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 460mg	20%
Total Carbohydrate 9g	3%
Dietary Fiber 4g	14%
Total Sugars 1g	
Includes 0g Added Sugars	0%
Protein 18g	
Vitamin D 1mcg	6%
Calcium 26mg	2%
Iron 2mg	10%
Potassium 530mg	10%

*The % Daily Value tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Protein content →

ROVITARIS® Binding Solution

Nutrition Facts	
servings per container	
Serving size	(100g)
Amount per serving	
Calories	200
<small>% Daily Value*</small>	
Total Fat 9g	12%
Saturated Fat 3g	15%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 460mg	20%
Total Carbohydrate 8g	3%
Dietary Fiber 3g	11%
Total Sugars 1g	
Includes 0g Added Sugars	0%
Protein 22g	
Vitamin D 1mcg	6%
Calcium 26mg	2%
Iron 2mg	10%
Potassium 530mg	10%

*The % Daily Value tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

← Protein content

Figure 5: The Nutrition Facts panel of samples made with 2% MC (left) and RBS (right). An increase in protein content was obtained in samples with 6% ROVITARIS® Binding Solution compared to samples made with 2% methylcellulose. Macronutrient values were confirmed through proximate analysis. Protein content was confirmed through Dumas method for total nitrogen.



Conclusion

Our patent pending ROVITARIS® Binding Solution powered by Rubi Protein™ stands out as a revolutionary solution for plant-based meat and seafood formulations. With low allergenicity and a premium nutritional profile, it's the ideal solution to address the clean label gap for replacing methylcellulose.

Leveraging the high solubility and gelling ability of Rubi Protein™, ROVITARIS® Binding Solution allows for targeted usage in plant-based meat and seafood products. During processing, it enables effective binding and easier handling and shaping by increasing viscosity at low temperatures. After processing, its ability to maintain binding and gelling properties across both hot and cold temperatures allows for a superior sensory experience through enhanced flavor, oil and texture retention.

ROVITARIS® Binding Solution powered by Rubi Protein™ has the potential to revolutionize the landscape of clean label plant-based meat and seafood products for both new product development and healthier reformulation. Its sustainability credentials are the final piece of the puzzle to satisfy consumers' desire for a 'better-for-you' clean label solution that does not compromise on taste and is beneficial for the planet. Making plant-based meat and seafood products more nutritional and tastier has never been easier.

Methylcellulose	ROVITARIS® Binding Solution
<input checked="" type="checkbox"/> Strong hot gel	<input checked="" type="checkbox"/> Strong hot gel
<input checked="" type="checkbox"/> Cold thickening	<input checked="" type="checkbox"/> Cold thickening
<input checked="" type="checkbox"/> Chemically modified additive	<input checked="" type="checkbox"/> Found and isolated from nature
<input checked="" type="checkbox"/> Fiber laxative	<input checked="" type="checkbox"/> Contains high quality protein
<input checked="" type="checkbox"/> Loss of gel at cold temperature	<input checked="" type="checkbox"/> Strong gel at cold temperature

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Plantible is a biology company that believes in a world where the transition to a more sustainable food system can be made more achievable when positive food choices don't lead to negative consequences. By unleashing the natural magic of RuBisCO protein, Plantible helps its customers and collaborators to transform their plant forward- and "free from"- foods to reach their full potential without compromise.

plantible

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