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January 21, 2014

Lisa M. Brines, Ph.D.  
National List Manager  
Standards Division, National Organic Program  
1400 Independence Ave, SW  
Room 2648-So., Ag Stop 0268  
Washington, DC 20250-0268

RE: National List Petition for inclusion of Whole Algal Flour to 7 CFR 205.606

Dear Ms. Brines,

Please find the attached response to the Subcommittee's questions, per the letter received on Dec. 11, 2011, regarding our whole algal flour product.

If you have any additional questions, please feel free to contact me.

Sincerely,

Teresa Chan  
Regulatory Affairs Manager



**CONFIDENTIAL BUSINESS INFORMATION STATEMENT**

Some of the information requested by the NOSB Handling Subcommittee contains confidential business information, in particularly, Question 1 - the request for Manufacturing Media on the full description of the media and nutrients. The materials used for our fermentation media and nutrients (Table 1) are trade secret information and if publicly disclosed, would cause financial and commercial harm to the company. A competitor, upon discovering our manufacturing raw materials would be able to quickly manufacture product with no investment in research or development, all to our company's detriment.



## Item B.5 – Manufacturing Process

1. Manufacturing media: Please provide a full description of the media used during the manufacturing/fermenting process, including a description of the source organism and nutrients added during the manufacturing process.

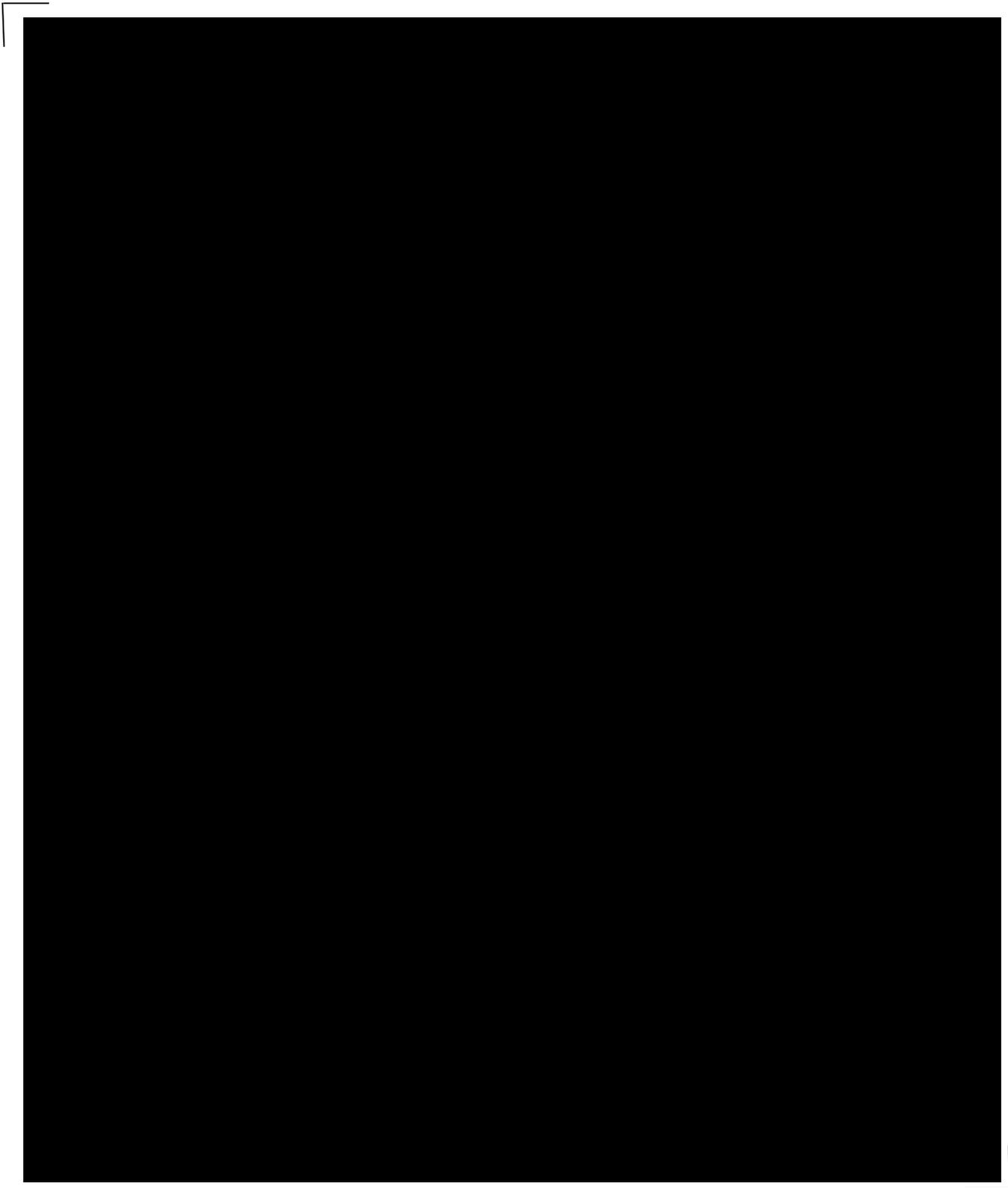
The source organism used to make Whole Algal Flour (WAF) is *Chlorella protothecoides*. As a species, *C. protothecoides* currently belongs to the genus *Chlorella* in the phylum Chlorophyta. Widespread in fresh and salt water, soil and air (Wu *et al.*, 2001), the green microalgae *Chlorella spp.* are nonmotile, unicellular eukaryotes, spherical in shape and have diameters that typically range from 2 – 10  $\mu\text{m}$  (Kay, 1991; Becker, 2007). The cell walls are hemicellulosic, rigid, and account for approximately 10% of the algal dry weight (Becker, 2007). *Chlorella spp.* also characteristically have membrane-bound organelles (Kay, 1991). Axenic<sup>1</sup> cultures of *Chlorella spp.* are easily established, in part because replication under optimum conditions tends to be rapid, often requiring less than two hours (Kay, 1991). Protein and lipid content and the fatty acid profile are known to vary widely with the species, the stage of cell growth (exponential or stationary), and with environmental conditions (*e.g.*, available nutrients, temperature, and light intensity) (Kay, 1991). As an example, the protein contents of *Chlorella spp.* have been reported to range from 15 – 60 % on a dry weight basis (Kay, 1991; Becker, 1994).

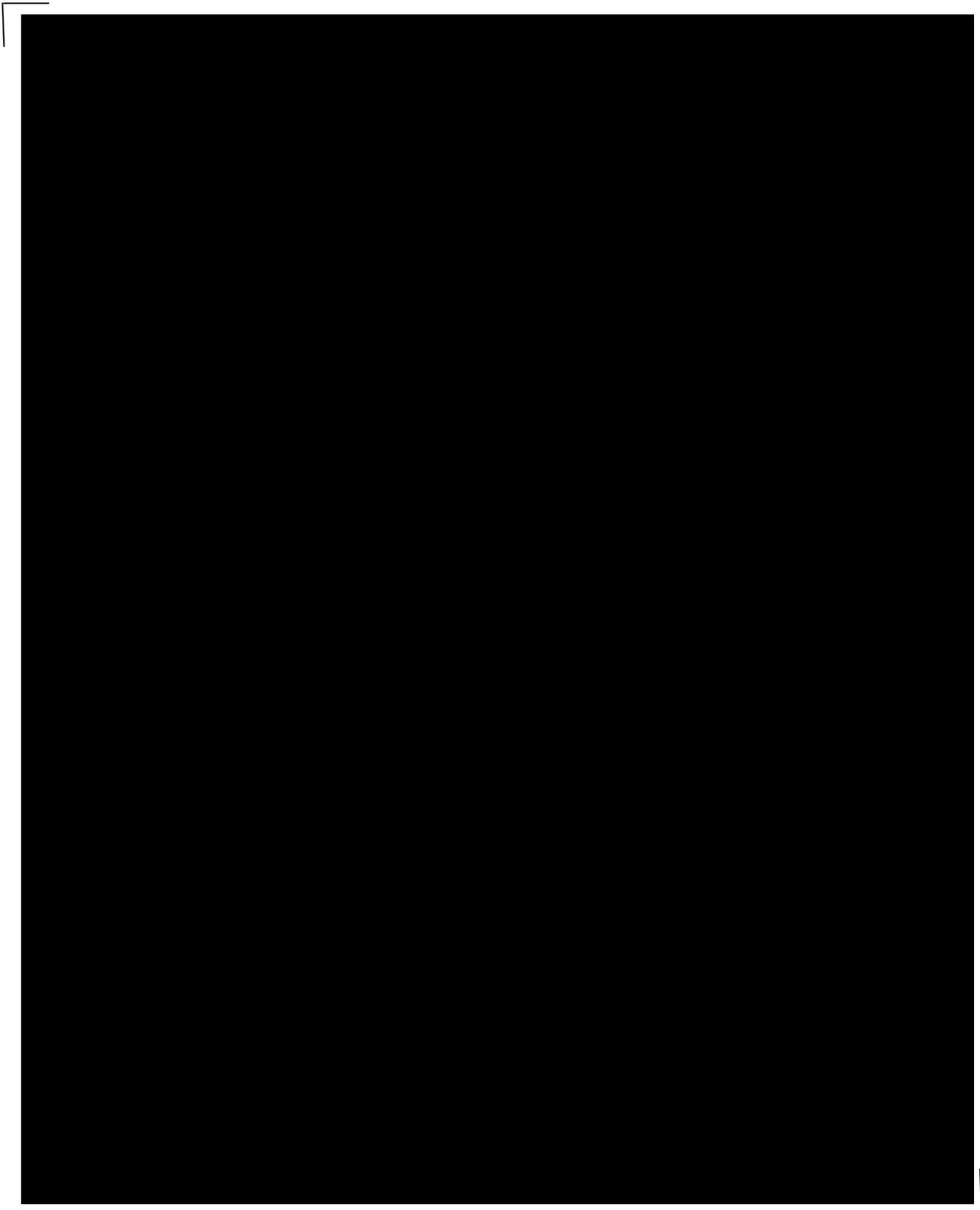
The ecophysiological and biochemical characteristics of *C. protothecoides* include the inability to utilize nitrate as a substrate, the need for thiamine but not for vitamin B12, the lack of secondary carotenoids (most strains) and a tolerance for high temperatures (28 – 34 °C upper limits), acidic pHs (3.5 – 4.0 pH) and high salt concentrations (3 – 4%) (Huss *et al.*, 2002). Morphology for the species includes the absence of pyrenoids, but the presence of a tri-laminar layer within the cell wall (Huss *et al.*, 2002). Reproduction is by endospore formation, an asexual internal process through which two to twelve sporangiospores are produced and released by each parental sporangium during vegetative growth (Ramirez-Romero *et al.*, 2010). It is worth noting that this growth pattern ceases when cells are deprived of nitrogen but continued to be fed sugar. Under nitrogen depleted conditions, the cell begins to synthesize and store copious amounts of oil (Day *et al.*, 2009).

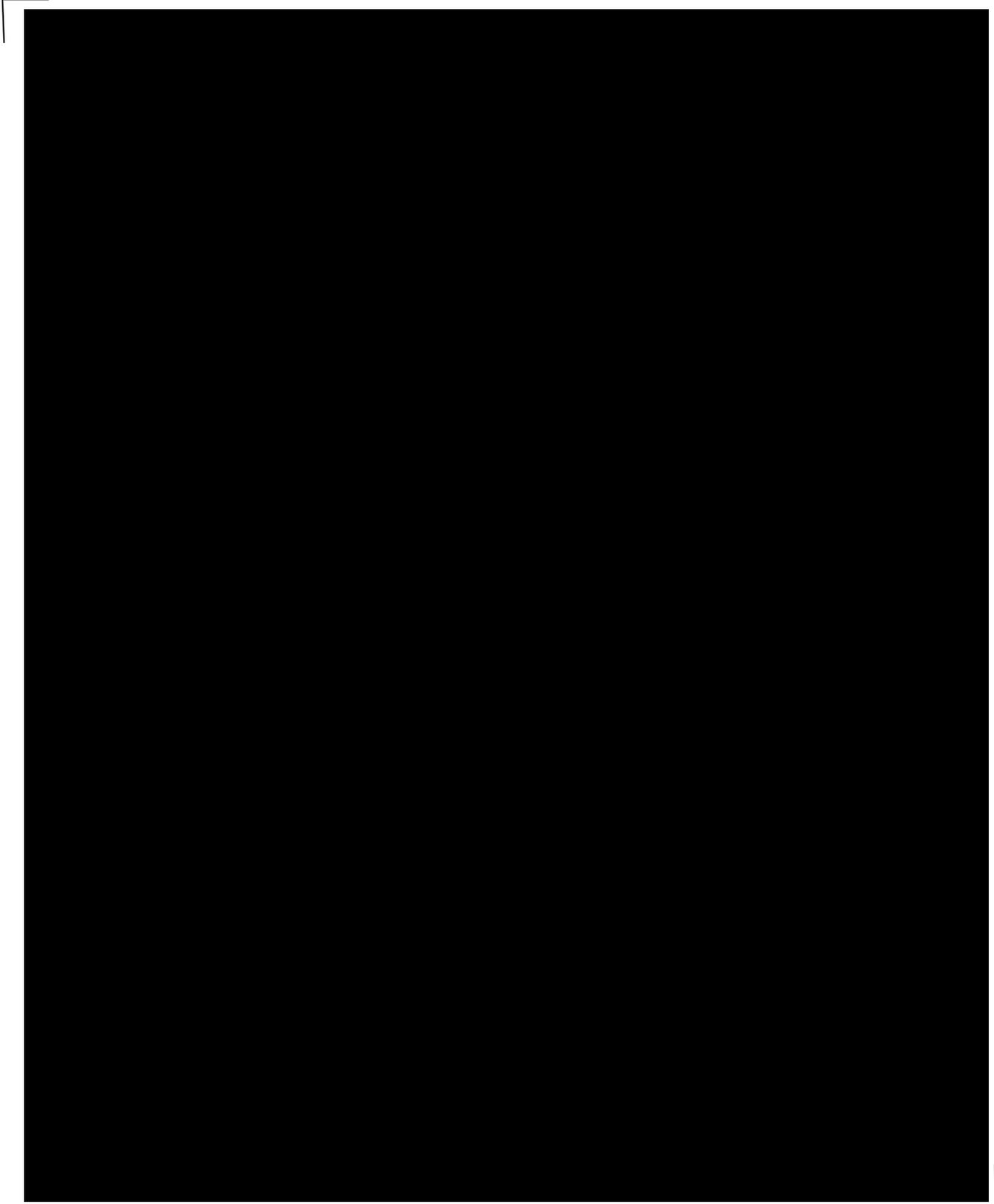
Whole Algal Flour is manufactured by fermenting and harvesting cultures of *Chlorella protothecoides*. Table 1, below, describes the media and nutrients used during fermentation and manufacture. These raw materials include industry standard fermentation media and nutrients used in other fermentation-derived food additives, such as gellan gum and xanthan gum, which are listed under 7 CFR 205.605(a) and 7 CFR 205.605(b), respectively.

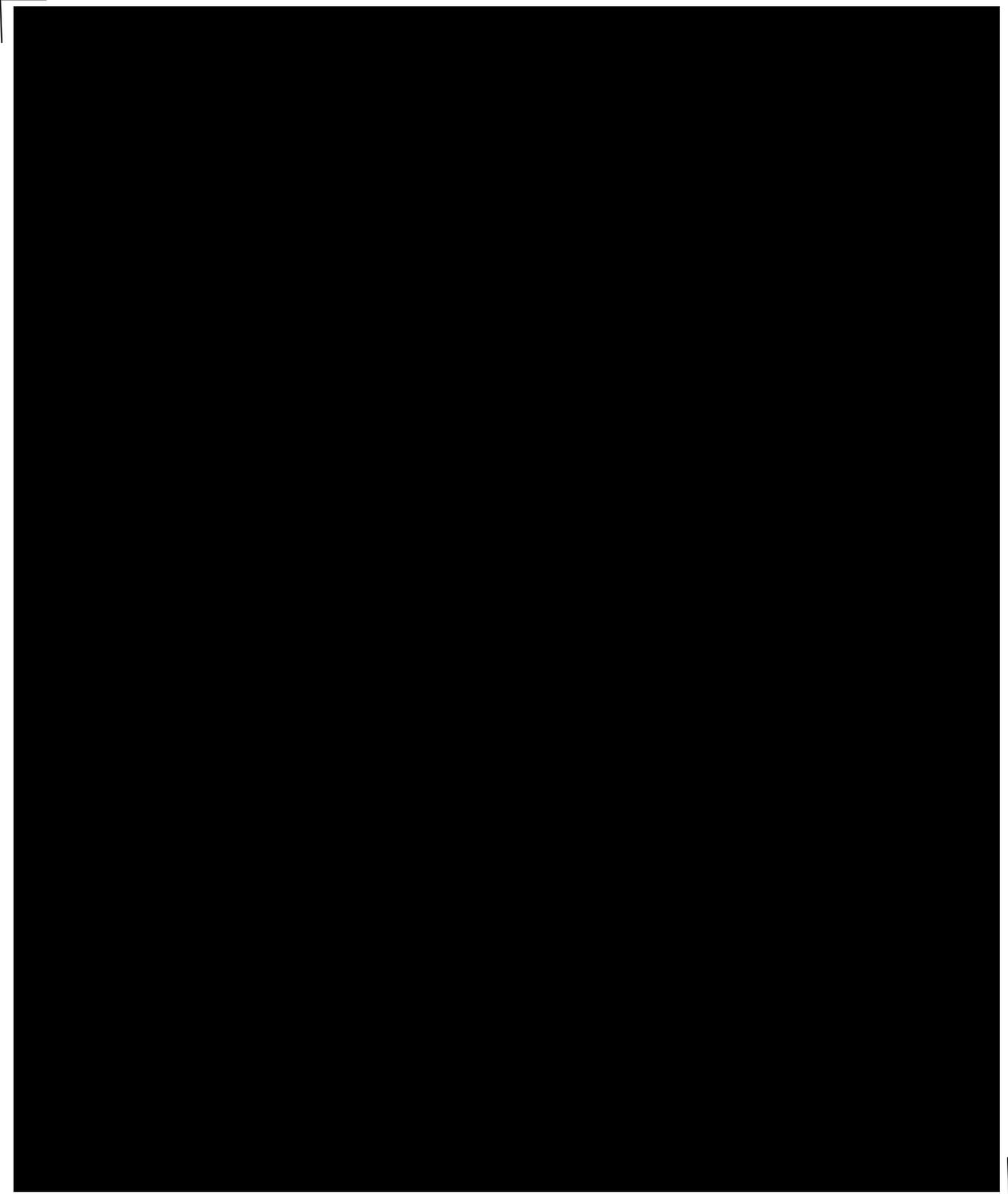
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<sup>1</sup> Cultures containing a single strains (i.e. not contaminated)











2. Antioxidants: The petition indicates that antioxidants are sometimes used during the production process. Are tocopherols the only antioxidant used for this purpose? If not, what other antioxidants are used? The petition also indicates that antioxidants are only needed for some applications. Please provide additional information on how the type or need for an antioxidant depends on the customer.

See Table 1, above, for the list of antioxidants used.

Antioxidants are added for customers where shelf life is a concern. Some customers may wish to use WAF immediately and do not wish to have any added antioxidants.

3. Ancillary Substances / Other Ingredients:

Other than food grade antioxidants, are there any other ingredients (e.g., flow agents, preservatives, solvents, etc.) used in the formulation of the finished product?

See Table 1, above, for the list of all materials used in the manufacture of WAF. If needed, food grade flow agents may be added to assist processing through drying and packaging; at present the use of such agents is not part of the standard process. Preservatives and solvents are not used in the manufacture of WAF, nor are they added to the finished product.

4. Genetically Modified Organisms:

The petition indicates that no genetically modified organisms are used in the production process. How is this verified during the process?

The organism used to produce WAF is *Chlorella protothecoides*. Solazyme's obtained this strain from the University of Texas (UTEX) Culture Collection of Algae in Austin, Texas. This strain is a pure, clonally isolated, non-genetically modified culture. To our knowledge, no GM version of this strain exists. Our production process isolates the strain and all WAF productions are made from a cryo-preserved working seed vial.